UNIVERSITY OF ALBERTA

Synthesis of *Trichinella spiralis* and *Candida albicans* Antigens, containing β-Mannopyranosyl Epitopes, their Conformation and Immunochemistry

BY

Mark Nitz ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Chemistry

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **"Synthesis of *Trichinella spiralis* and *Candida albicans* Antigens, containing β-Mannopyranosyl Epitopes, their Conformation and Immunochemistry"** by **Mark Nitz** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**.

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Abstract

The chemistry and immunochemistry of a parasite glycoprotein antigen and a yeast cell wall polysaccahride antigen, both of which contain a β-mannopyranosyl residue, have been investigated by synthesis, molecular modelling and by quantitation of the immune response.

The first oligosaccharide (**3**) occurs in the *N*-linked glycan of *Trichinella spiralis*, a pathogenic nematode that causes trichinosis. The glycan (**3**) contains a unique capping 3,6-dideoxy-β-D-*arabino*-hexopyranoside (Tyvelose, Tyv) and a LacDiNAc [β-Gal*p*NAc-(1→4)-β-Glc*p*NAc] element.

The glycan was synthesized as the linear trisaccharide functionalized for glycoconjugate formation and then elaborated enzymatically to the branched tetrasaccharide (**3**). Numerous attempts to introduce the capping 3,6-dideoxy-β-D-*arabino*-hexopyranoside using intramolecular aglycon delivery were attempted. Finally, an efficient synthesis was achieved via introduction of the dideoxyhexose residue as a β-D-*ribo*-hexopyranoside (paratose) followed by an oxidation-reduction sequence to generate the β-D-*arabino* configuration. The required dideoxyhexose donor was synthesized in a series of high yielding steps from glucose utilizing the *p*-methoxyphenyl-glycoside. The α(1→3)fucosyltransferase V efficiently introduced the branching fucose residue to the linear trisaccharide.

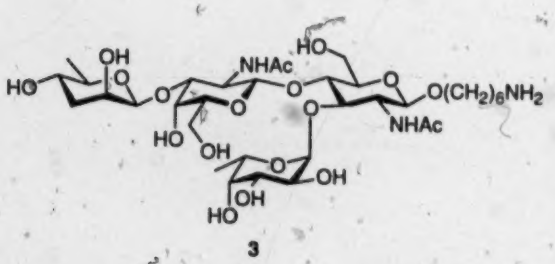
The inhibitory binding studies involving a monoclonal antibody isolated from a *T. spiralis* infected rat and the synthetic linear oligosaccharide (**3** lacking the fucose residue) suggests that the 3,6-dideoxy-β-D-*arabino*-hexopyranoside most likely exists on a novel and yet to be determined glycan structures.

Progress toward the synthesis of β-manno type linkages opened the possibilities for the synthesis of a portion of the (1→2)-β-mannopyranan, found in the phosphomannan of the

pathogenic fungus *Candida albicans*. Oligomers of this antigen were synthesized using a novel approach, which employed a *p*-chlorobenzyl protected ulosyl bromide as the glycosyl donor in a participating solvent. This synthesis provided the trisaccharide through hexasaccharide structures for conformational and immunochemical investigations (**89-92**). Tethered versions of the tetrasaccharide and hexasaccharides were also synthesized for use as synthetic antigens when covalently attached to bovine serum albumin and tetanus toxoid. A mimetic of (1→2)-β-mannopyranotetrose containing a terminal thioglycosidic linkage was also synthesized.

The synthetic (1→2)-β-mannopyranans exhibited an exceptional number of long range NOE contacts spanning N to N+3 residues. Quantitative interpretation of the NOE data in conjunction with molecular dynamics simulations showed the polysaccharide antigens to occupy a unique compact helical structure, with a trisaccharide repeating structure corresponding to the immunochemical epitope.

The tetanus toxoid conjugates of the (1→2)-β-mannopyranans induced a strong antibody response against isolated *C. albicans* polysaccharide in rabbits. Inhibition studies with mouse monoclonal antibodies, known to be protective against *C. albicans*, were found to bind the propyl (1→2)-β-D-mannotriose (**89**) with an affinity significantly higher than larger oligomers. This unprecedented inhibition data is rationalized with the conformational model of the anitgen and suggests that a synthetic trisaccharide conjugate vaccine may provide protection against *C. albicans*.



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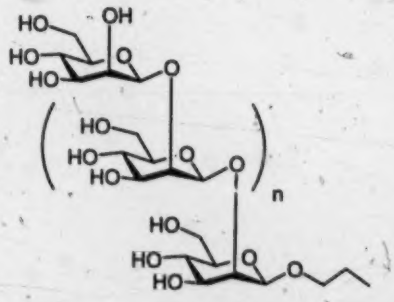

n=1-4 **89-92**

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Abbreviations

Ac	acetyl
All	allyl
AMBER	assisted model building with energy refinement
Asn	asparagine
Bn	benzyl
BSA	bovine serum albumin
Bz	benzoyl
CAc	Chloroacetate
2D	two dimensional
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
ES	excretory/secretory
ES HRMS	electrospray high resolution mass spectrometry
ESMS	electospray mass spectrometry
Et	ethyl
GCOSY	gradient coupling correlated spectroscopy
GalNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-D-galactose
GC/MS	gas chromatography/Mass spectrometry
Glc	glucose
GlcNAc	*N*-acetylglucosamine, 2-acetamido-2-deoxy-D-glucose
GlcNPhth	*N*-phthaloylglucosamine, 2-deoxy-2-phthalimido-D-glucose
h	hour
HMQC	heteronuclear multiple quantum coherence
HPLC	high performance chromatography
HRP	horseradish peroxidase
IAD	Intramolecular aglycon delivery

IC_{50}	inhibitor concentration required giving 50% inhibition
LacDiNAc	2-deoxy-2-acetamido-β-D-galactopyranosyl-(1-4)-2-deoxy-2-acetamido-β-D-glucopyranose
MAb	monoclonal antibody
MALDI-TOF	matrix assisted laser desorption ionization time of flight
Man	mannose
MD	molecular dynamics
Me	methyl
MHC	major histocompatibility complex
NBS	*N*-bromosuccinimide
NeuNAc	5-Amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid
NIS	*N*-iodosuccinamide
NMR	nuclear magnetic resonance
PBS	phosphate buffer saline
PBST	phosphate buffer saline containing Tween 20
Ph	phenyl
PhthN	phthalimido
ppm	parts per million
Pyr	pyridine
ROE	rotating-frame nuclear Overhauser effect
rt	room temperature
S_N2	bimolecular nucleophilic substitution
TBAF	tetrabutylammonium fluoride
TBDPS	*t*-butyldiphenylsilyl
Tf	trifluoromethanesulphonate
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TMB	3,3',5,5'-tetramethylbenzidine
TMSOTf	trimethylsilyl trifluoromethanesulfonate
T-ROESY	transverse rotating-frame Overhauser effect spectroscopy
TT	tetanus toxoid
UV	ultraviolet

Chapter 1

Introduction

Oligosaccharides and their Synthesis

1.1 Importance, and types, of carbohydrates in biological systems

Using a combination of analytical techniques, organic synthesis and molecular biology, new functions of carbohydrates are routinely being discovered in a field known as glycobiology.[1] It has been found that many events that require intercellular interactions such as fertilization, embryogenesis, bacterial and viral infection, neuronal development, the proliferation of cells and their organization into tissue types, hinge upon oligosaccharide based interactions.[2,3] The carbohydrates that mediate these events are generally found as part of a glycoprotein or glycolipid.

Glycoproteins can further be classified into *O*-linked or *N*-linked depending on the type of linkage to the reducing sugar of the glycan. In the *N*-linked glycans, 2-acetamido-2-deoxy-D-glucose (GlcNAc) is linked via an *N*-glycosidic bond to the amide of asparagine residues. The asparagine residue is usually found in the Asn-Xxx-Thr (where Xxx is not proline) consensus sequence, although this sequence is not enough to ensure glycosylation.[4,5] This GlcNAc residue exists as the reducing terminus of a common pentasaccharide found at the core of all *N*-linked glycans (Figure 1.1). The core structure is elaborated to form the wide variety of *N*-linked glycoproteins found in eukaryotic organisms.[6]

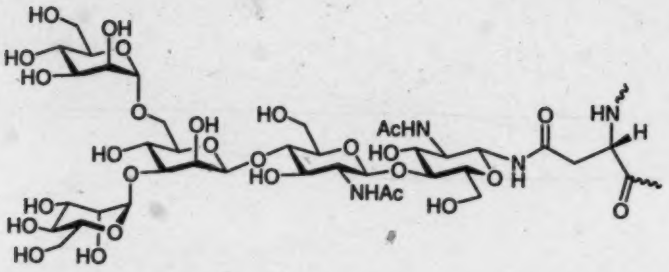


Figure 1.1 The structure of the core pentasaccharide found in *N*-linked glycans

Figure 1.2 gives a representative example of an *N*-linked glycan, which indicates the potential size and complexity of these structures. This glycan is capped by a single sialyl Lewis-x structure that is known to be involved in leukocyte recruitment.[6]

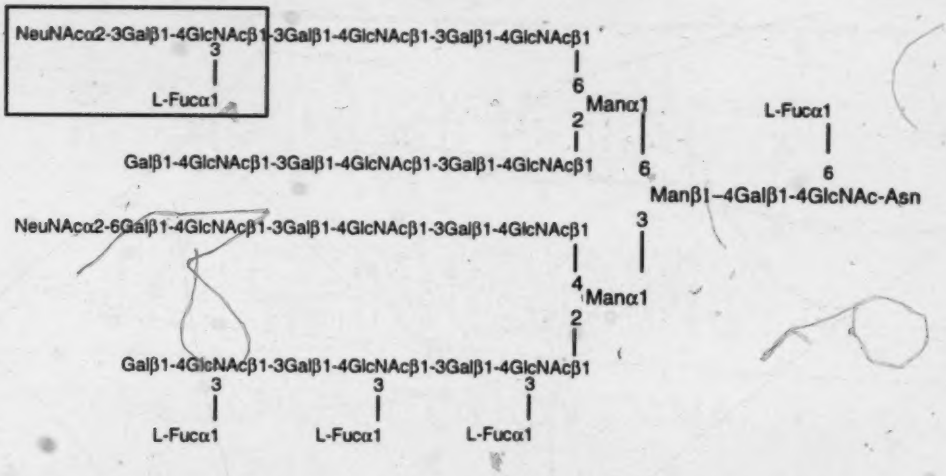


Figure 1.2. Tetra-antennary glycan isolated from human granulocytes. Box denotes the Sialyl Lewis-x epitope

O-linked glycans were initially isolated from mucus where they are linked directly to the serine or threonine amino acids of mucin proteins. They have now been discovered in a wide variety of cell and tissue types. α-D-Galactosamine[7] is usually the reducing sugar in these glycans although other sugars such as mannose[8], fucose[9], xylose[10], galactose[11] and 2-acetamido-2-deoxy-glucose[12] have been found in specific instances. The *O*-linked glycans are much more heterogeneous than *N*-linked glycans, containing at least five different core structures, but they

are usually smaller and less elaborate than the *N*-linked glycans.[13] *O*-Linked glycans often exist at higher densities than *N*-Linked glycans forming large hydrophilic domains on membrane proteins that are thought to protect the surfaces of some cell types. The recent association of *O*-linked glycans with the structures recognized (epitopes) by cancer specific antibodies has led to increased interest in their properties.[14]

In the glycolipids a β-D-glucopyranosyl residue is linked to ceramide, which anchors the structure in the membrane of its host cell. The reducing β-D-glucopyranosyl moiety is elaborated with β-D-galactose to form a lactosyl core, which is further elaborated to give a ganglioside or glycosphingolipid structure. The glycosphingolipids can carry the Lewis or blood group determinants on one of four core structures that vary with tissue type. The gangliosides contain one or more residues of *N*-acetylneuraminic acid and have been associated with some tumor epitopes.[15]

The increasing efforts to study carbohydrate mediated events in detail have been difficult due to the inherent challenges of working with the glycans involved. They have inherently low affinity for the proteins mediating their binding events, and depend heavily on multivalency in their interactions.[16] Native structures are usually only available in minute quantities, and there exists no amplification or automated synthesis procedures for their preparation. Due to these difficulties many of the studies of carbohydrates in biological systems have relied on indirect methods, such as anti-carbohydrate antibodies, to localize and characterize the carbohydrate mediated processes. It is preferable to have defined carbohydrates and carbohydrate conjugates to directly probe these interactions. These compounds are only available through chemical synthesis, which will continue to play a key role in glycobiology providing access to new carbohydrate structures and their mimetics.

1.2 Synthesis of oligosaccharides

1.2a Introduction

The chemical synthesis of oligosaccharides is more complicated than the synthesis of other biopolymers such as protein and DNA. This is due to the large number of possible combinations of monomers and the variability of the functionality found within them. There are no universally applicable methods for the synthesis of glycosidic linkages, and an extensive literature of different methods for specific cases attests to this difficulty.[17,18] Despite these challenges, the development of convergent strategies that integrate glycosylation methods with versatile protecting groups have facilitated the synthesis of oligosaccharides containing upwards of 20 residues.[19]

The most common disconnection for the retrosynthetic analysis of glycosides involves breaking the acetal carbon oxygen bond to the aglycon. Synthetically this is accomplished by activating a leaving group at the anomeric centre to allow attack of the aglyconic alcohol with concurrent formation of a glycosidic linkage. Traditionally glycosyl halides have been the most commonly employed anomeric leaving group, as they are readily synthesized and easily activated with many combinations of different heavy metal salts.[20] Unfortunately these compounds are notoriously unstable and often require strongly acidic conditions for their synthesis, limiting their utility. Recently there has been an explosion in different types of anomeric leaving groups and methods to activate them but only three have become widely accepted (Figure 1.3).[17] Trichloroacetimidates,[21] thioglycosides[22] and glycosyl sulfoxides[23] are now used in the majority of oligosaccharide syntheses. Trichloroacetimidates can be introduced under mild conditions late in a synthesis, in the presence of most protecting groups, and have found extensive use as leaving groups for oligosaccharide donors.[24] Thioglycosides are extremely versatile since they are stable to most protecting group manipulations and therefore serve as anomeric protecting groups that can be activated as leaving groups. They can also be oxidized to sulphoxides that serve as even more powerful leaving groups that have found use in difficult glycosylations.

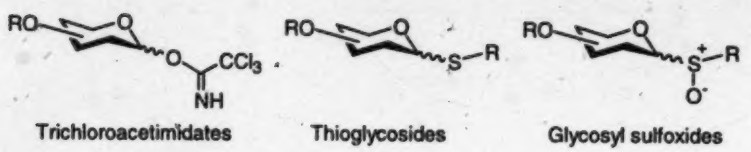


Figure 1.3. Common leaving groups employed for glycoside synthesis

For synthetic purposes it is convenient to divide glycosidic linkages into three common classes based on their stereochemistry and relationship to the functional group at C-2 of the sugar ring: 1,2-trans α and β, 1,2-cis α and 1,2-cis β (Figure 1.4). Each class has a general approach by which it is synthesized. Other linkages do occur in oligosaccharides, such as α-sialic acid and 2-deoxy glycosides, but specialised methods have been developed to produce these linkages.

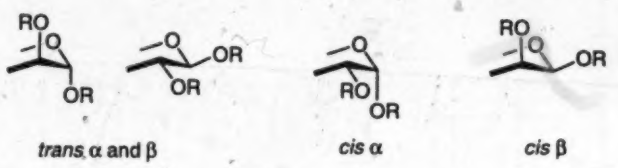


Figure 1.4 Classification of glycosidic linkages

1.2b Formation of 1,2-trans-α and β linkages

Trans α and β linkages are the most easily synthesized, although many exceptions exist with these highly functionalized molecules. Their synthesis usually relies upon a participating acyl group at C-2 that is able to direct the incoming alcohol in a trans orientation via an acoxonium ion intermediate. Transient formation of an orthoester can occur by attack of the incoming alcohol at C-2 of the dioxolane ring. Under acidic reaction conditions this product rearranges to give the 1,2-trans glycoside product (Figure 1.5).[25]

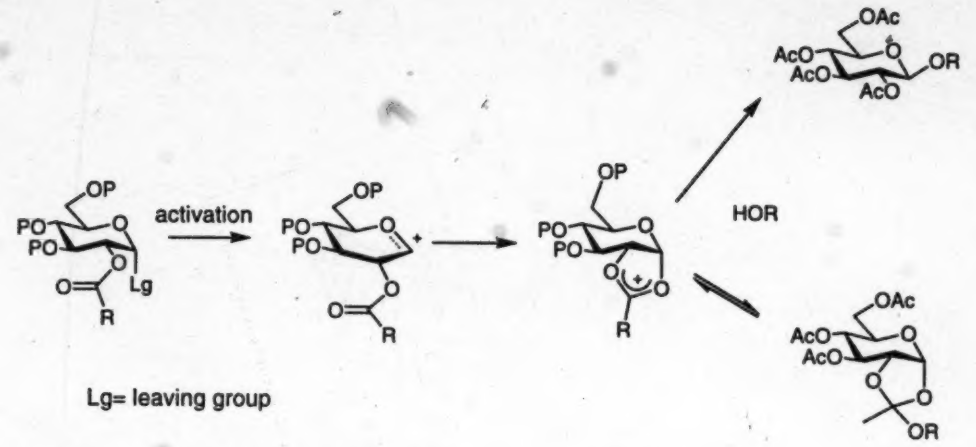


Figure 1.5. Proposed mechanism for participating group directed glycosylation

It is also possible to generate an equatorial glycosidic bond in the absence of a participating group using a participating solvent, generally a nitrile. This observation was reported independently by many groups and was explained in terms of the generation of an α-nitrilium ion intermediate, which is then displaced by an incoming alcohol (Figure 1.6).[26,27] Generally, modest to high selectivities for the equatorial glycoside can be obtained. This approach has been particularly advantageous for *N*-acetylneuraminic acid donors that lack the functionality necessary to install a participating acyl group.[28]

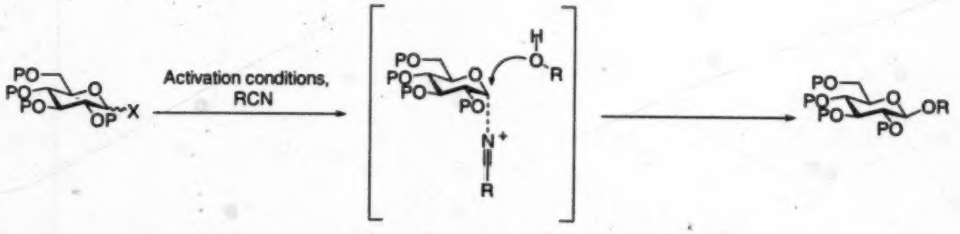


Figure 1.6. Proposed mechanism to explain equatorial selectivity of nitrile solvents

1.2c Formation of 1,2-cis-α linkages

1,2-cis-α glycosides are synthesized by employing a non-participating group, generally an alkyl group, adjacent to the anomeric position. Their synthesis takes advantage of the relative energies of the transition states involved in the formation of α and β-D-glycosides in order to control the stereochemistry about the anomeric center.

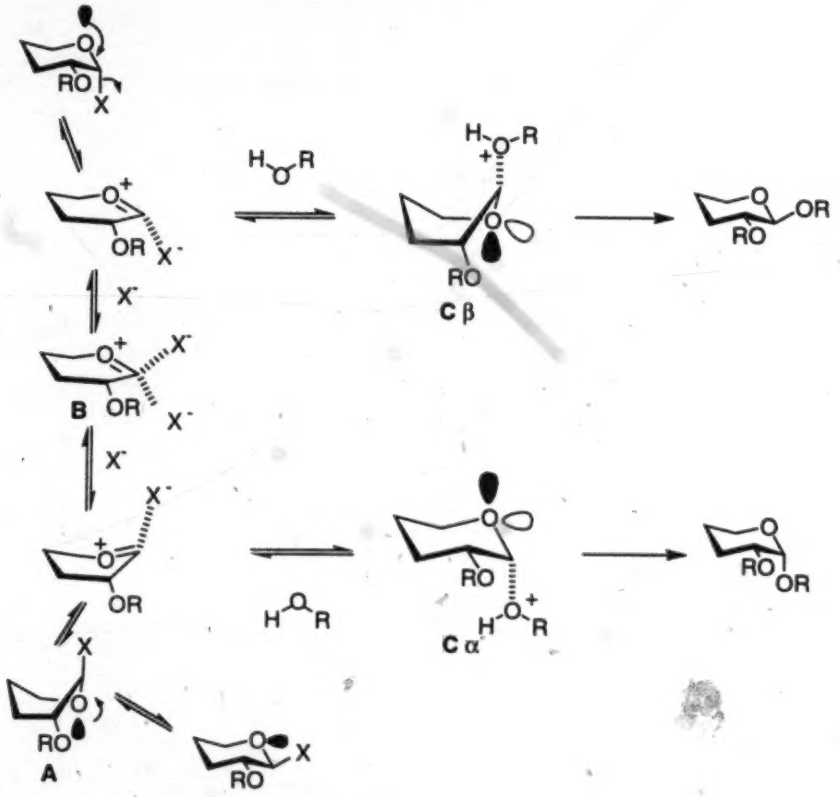


Figure 1.7. Proposed mechanism of halide-ion promoted α-glycoside formation

For maximal charge delocalization about the anomeric carbon, it is necessary to have an unshared pair of electrons on the ring oxygen atom antiperiplanar to the geminal polar bond (shown shaded, Figure 1.7). This property generally manifests itself as the anomeric effect. Thus, for stabilisation of the polar transition states, a change in conformation of the pyranose ring

to the proposed ^{1,4}**B** conformation is necessary as the β-halide in structure **A** leaves, or as the β-glycoside **C** β forms (Scheme 44). It is hypothesised that the transition states leading to the glycosides, **C** α and **C** β, are the highest in energy due to the lack of the strong stabilising ionic interaction with the negatively charged counter ion present in the other intermediates. However, the transition state **C** α, leading to the α-glycoside, is lower in energy than **C** β because of its preferred chair conformation, favouring α-glycoside formation. The activating conditions used must establish rapid conversion between α- and β- glycosyl halides via the ion triplet **B** that maintains equilibrium between the two transition states **C** α and **C** β leading to glycoside formation. Given that the isomerization between α and β intermediates is fast in comparison with the glycosylation step, and the difference in stability between the two transition states, **C** α and **C** β is large enough, the reaction can be steered to generate the α-glycoside.[29]

1.2d Formation of 1,2-cis-β-D-glycosides

The final class of compounds, 1,2-cis-β-glycosides, is the most challenging to synthesize. Neither participating groups nor the anomeric effect can be used to facilitate the formation of this type of linkage. Traditionally the use of α-glycosyl halides and an insoluble silver supported catalyst has been used to direct the glycosylation to the β-face of the pyranose ring.[30] It is hypothesized that with these catalysts activation of the leaving group occurs at the solution-solid interface. Thus, when the glycosyl donor is activated, the solid supported catalyst blocks the α-face and the glycosylation is directed to form the β glycoside (Figure 1.8). This method works well for activated alcohols but fails to give good selectivity when used with many common glycosyl acceptors.

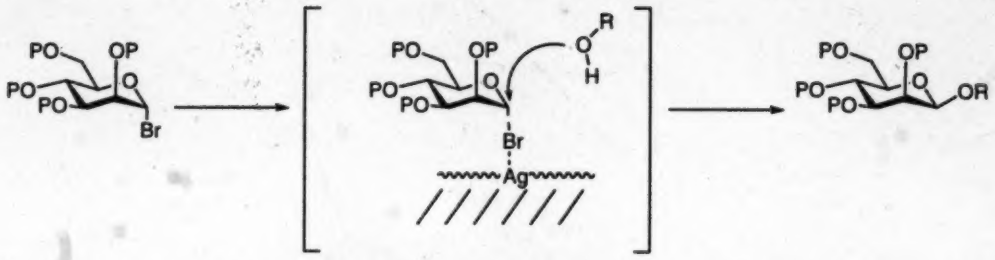


Figure 1.8. The use of a solid supported catalyst in the formation of 1,2-cis-β-glycosides

Many chemists have turned to more reliable methods for the synthesis of this type of glycoside involving the epimerization about C-2 after the formation of the glycoside. Thus, neighboring group participation is used to first form the 1,2-trans glycoside, a relatively simple condensation in comparison, and then C-2 is epimerized. Epimerization at C-2 has been achieved through two routes; an oxidation to the ulose followed by reduction or activation to a good leaving group and an S_N2 displacement (Figure 1.9).

Oxidation and reduction has been used successfully to convert many glucosyl oligosaccharides to the mannosyl derivatives. The oxidation step is usually successfully carried out with acetic anhydride in DMSO[31,32] and reduction can be carried out with sodium borohydride in mixed solvent systems.[33,34] For more difficult reductions L-selectride has been applied with good results.[35]

Nucleophilic inversion at C-2 has been accomplished intra and inter-molecularly. In these cases it is necessary to have a 4,6-*O*-benzylidene protecting group present to prevent ring contraction reactions that can occur when a good leaving group is introduced at C-2. Triflates are typical leaving groups for these reactions, used in conjunction with the tetrabutylammonium salt of the desired nucleophile,[36] or more recently the cesium salt under ultrasound promotion.[37]

Günther and Kunz have epimerized C-2 via an intra-molecular reaction using a carbamate protecting group at C-3 to displace the triflate at C-2.[38] This reaction gives high yields of the expected 2,3-cyclic carbonate after a mild acid hydrolysis of the iminocarbonate generated by the

displacement. Unfortunately the extra participating group manipulations to synthesize the carbamate protected glycosyl donor reduce the generality of this type of synthesis.

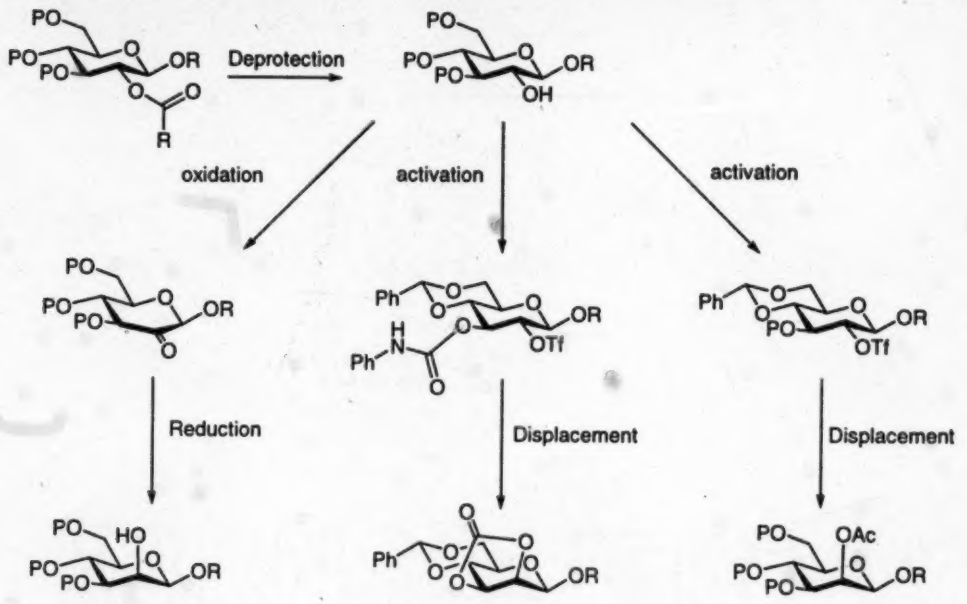


Figure 1.9. Methods for synthesis of 1,2-cis-β-glycosides via C-2 epimerization

All of the methods outlined thus far for the synthesis of 1,2-cis-β-glycosides suffer from a lack of anomeric selectivity, or require extra manipulations before or after glycoside synthesis, which limit the efficiency of these transformations. For this reason there has been a significant amount of research into forming 1,2-cis-β-glycosides efficiently in a manner that does not require manipulation after glycoside synthesis.

1.3 Recent advances in the formation of 1,2-cis-β-glycosides

1.3a Intramolecular aglycon delivery

The numerous attempts at 1,2-cis-β-glycoside synthesis had demonstrated that it was not likely that the traditional intermolecular approach would lead to the selectivity desired for their

synthesis. Barressi and Hindsgaul, and, Stork and Kim independently investigated intramolecular aglycon delivery (IAD) to form β-D-mannosides (Figure 1.10). This approach uses a tethering element (X) to link the glycosyl donor and acceptor prior to glycoside synthesis. Upon activation of the latent glycosyl donor, the glycosyl acceptor is delivered to form the desired 1,2-cis-β-glycoside. For this type of synthesis to be successful it is necessary to use a tethering element (X) that can link the glycosyl acceptor and donor in high yield. After activation of the glycosyl donor the tethering element must then be able to stabilise the positive charge generated as the aglycon leaves. Preferably the tether is also hydrolysed upon quenching of the reaction.

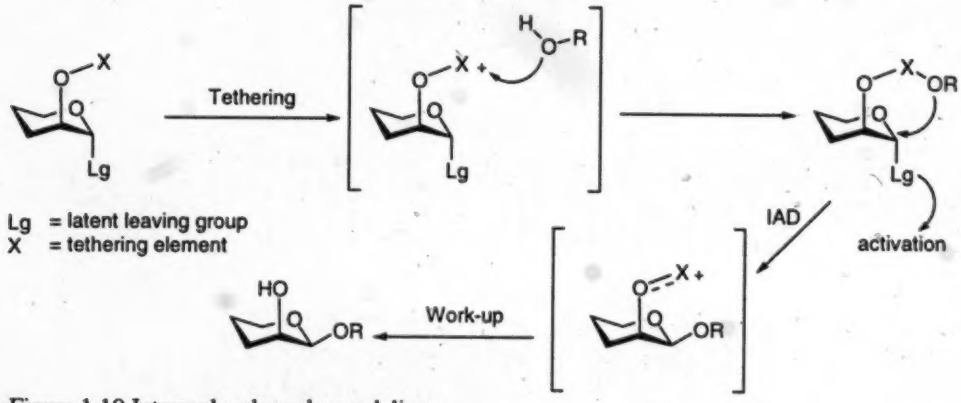


Figure 1.10 Intramolecular aglycon delivery

Hindsgaul and Barressi first published the use of a dimethyl acetal to prearrange the glycosyl donor and acceptor (Figure 1.11).[39,40] The dimethyl acetal has the advantage of being a symmetrical linkage which avoids the possibility of diastereomeric acetals which may react differently during the IAD. This linkage was cleverly synthesized in a two step procedure from a 2-*O*-acetate via functional group conversion with Tebbe's reagent,[41] to give an enol ether, followed by acetal formation with the aglyconic alcohol under acidic catalysis. Upon activation of the glycosyl donor an intramolecular reaction occurs delivering the aglycon to the β-face of the glycosyl donor, resulting in a 1,2-cis-β-glycoside after quenching with water. Recently the use of

NIS has allowed the tethering and intramolecular glycosylation to be achieved in one pot.[42] NIS is used to activate the enol ether resulting in an 1-iodo-2,2-propyl acetal tether. Upon warming the thioglycoside was activated by a second equivalent of NIS and the intramolecular reaction occurs. This improvement has not been extensively investigated, having only been reported with a limited number of alcohols.

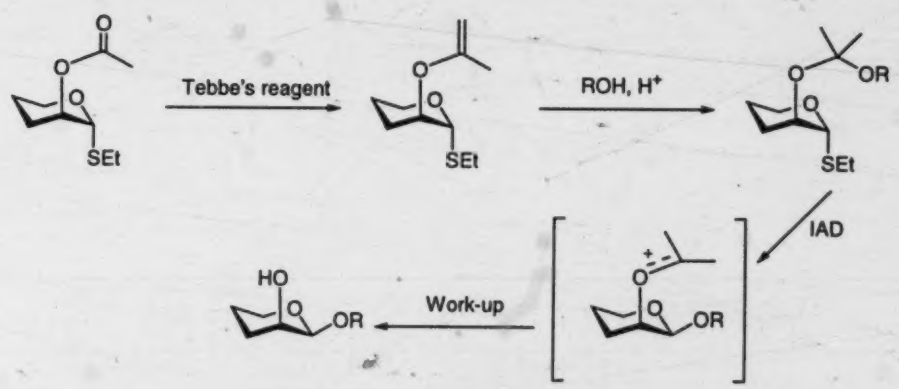


Figure 1.11. Intramolecular aglycon delivery using a 2,2 propyl acetal tether

Stork *et al.* relied upon the use of a dimethyl silyl acetal as a tethering element (Figure 1.12).[43,44] Using dichlorodimethylsilane it was possible to join the glycosyl donor and acceptor in a single step by stirring the three components in equimolar ratios.

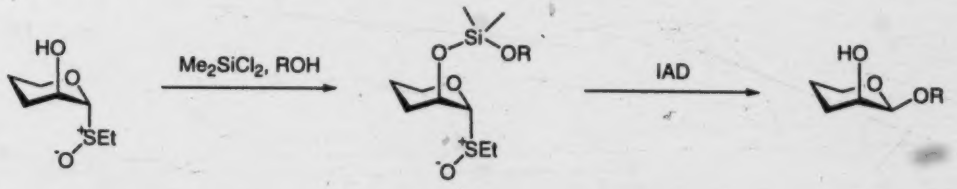


Figure 1.12. Intramolecular aglycon delivery using a silyl acetal tether

The latent glycosyl donor was then activated to give the desired linkage. The best yields were obtained when a glycosyl sulfoxide was used as a glycosyl donor. Bols has extended the use of

these tethers to form 1,2-cis-α glycosides using the respective thioglycosides as glycosyl donors.[45]

A recent development in IAD methodology was the introduction of an efficient *p*-methyoxybenzidene acetal tether by Ito and Ogawa (Figure 1.13).[46] Taking advantage of the commonly used *p*-methoxybenzyl ether protecting group, oxidation with DDQ in the presence of an alcohol forms a *p*-methoxybenzidene acetal. This tether should be more active than previous tethers because of the formation of a highly stabilized carbocation upon glycosylation. Unfortunately, the acetal is also too labile to be isolated by standard silica gel chromatography necessitating running the two reactions sequentially without purification of the intermediate acetal. Surprisingly good yields of β-mannosides have been achieved over these two steps in specific cases. The synthesis of *N*-linked core derivatives have been accomplished, attesting to the efficiency with which the β-D-mannopyranosyl(1→4)-β-D-2-deoxy-2-acetamido-glucopyranosides can be formed using this approach.[47,48]

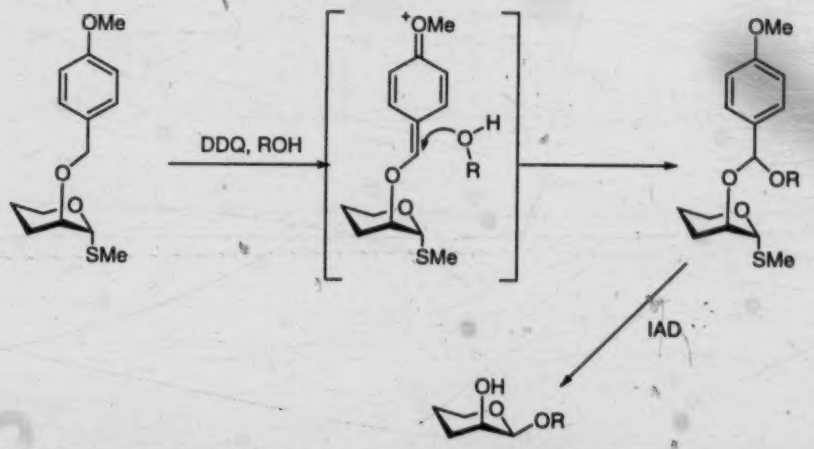


Figure 1.13. Intramolecular aglycon delivery using a *p*-methoxybenzyl acetal tether

Significant efforts have been expended to understand and optimise this reaction. It was found that the presence of a rigid bridging 4,6-*O*-benzylidene or 4,6-*O*-cylcohexylidene favours the collapse of the scaffold to the desired glycoside. The presence of a large protecting silyl group at C-3 also improves the yields of these reactions.[49] Investigation of the acetal diastereomers

formed during the oxidation of the *p*-methoxybenzyl ether has shown that the reaction is usually diastereoselective, giving the R or S isomer depending on the compounds brought together to form the acetal. Surprisingly both diastereomers were equally efficient in the IAD process.[50] Further development of this reaction has used a solid phase linked tether, which allows purification of the acetal intermediate through washing of the solid phase.[51]

Other tethering approaches have also been used. These involve tethering with an element that is not destroyed by the glycosylation reaction, thus leading to a macrocyclic structure. The steric and torsional constraints of the macrocycle are responsible for controlling the stereochemistry of the glycosylation. The succinyl and malonyl bridged glycosyl donors and acceptors have given high selectivity for the formation of certain β-mannosides. It is difficult to predict which combinations of tether length and linkage positions will give the desired glycoside and this remains a trial and error endeavour.[52,53] For example, when the 2-*O*-mannosyl-4-*O*-glucosyl succinyl tether is used a 2:1 α:β ratio of glycosides is formed, but when a 6-*O*-mannosyl-6-*O*-glucosyl succinyl tether is used exclusively, the β-glycoside is formed (Figure 1.14).

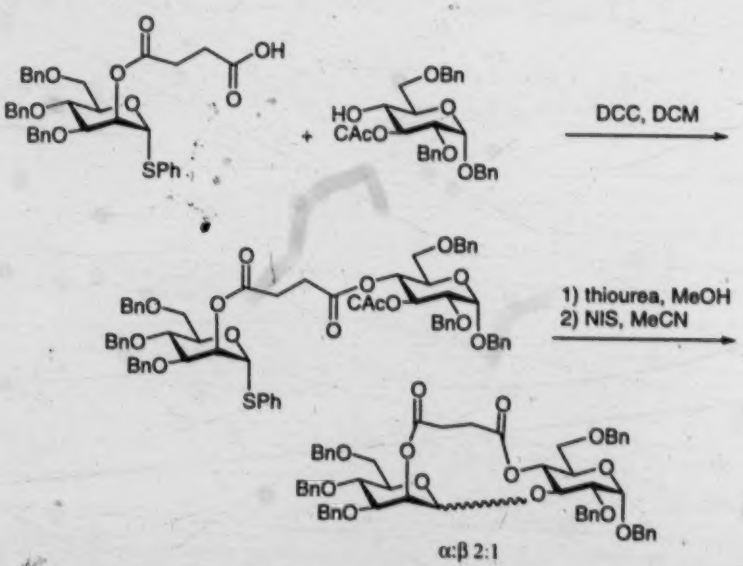


Figure 1.14. Use of succinyl tether for formation of 1,2-cis-β-glycosides

This approach is not as efficient as the IAD approach as it requires multiple protecting group manipulations to obtain the tethered glycosides, making it poorly amenable to the synthesis of oligosaccharides.

1.3b Other recent approaches

As an extension of the oxidation and reduction approach used for the synthesis of β-mannosides the use of ulosyl bromide donors has been developed.[54] These donors have the advantage of eliminating a deprotection and oxidation step necessary after the formation of the glycosidic linkage, increasing the efficiency of this approach. In order to direct the glycosylation in the equatorial orientation a heterogeneous catalyst is used to activate the α-ulosyl bromide blocking attack from the axial direction (Figure 1.15). Once the glycosylation has been accomplished selective reduction is possible to give the desired β-mannosides. Few accounts of the use of these donors with deactivated alcohol acceptors have been published; indicating the application of this reaction may be limited to active glycosyl acceptors.[55,56]

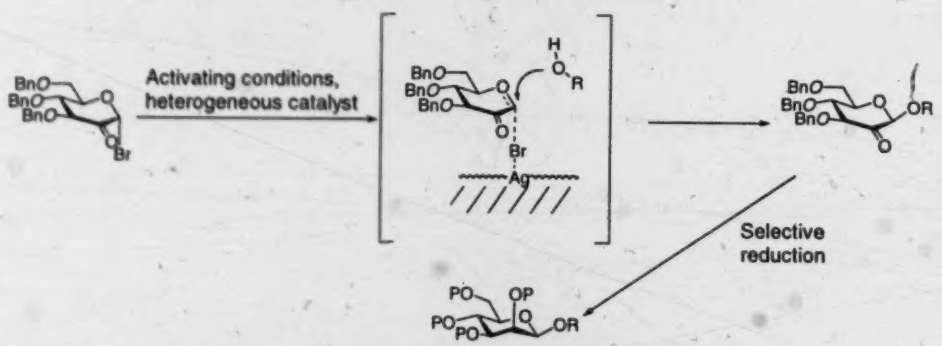


Figure 1.15. Use of ulosyl bromides for the formation of β-mannosides

Recently a thorough investigation of glycosylations with mannosyl sulfoxides has shown that under certain conditions specifically protected mannosyl sulfoxides can lead to high yields of β-mannosides via a glycosyl triflate (Figure 1.16).[57] Provided that this triflate is stable under the

reaction conditions, an S_N2 displacement at the anomeric centre by the acceptor alcohol nucleophile can be achieved, giving a β-mannoside. To stabilise the anomeric triflate it is necessary to employ a 4,6-*O*-benzylidene protecting group on the glycosyl donor. This suppresses flattening of the pyranose ring that can lead to oxocarbenium ion formation and loss of selectivity.[58] Unfortunately this reaction only gives high selectivities and yields with active alcohols, while the relatively unreactive 4-OH of glucose gave a 31 % yield in a 3.8:1 β:α ratio.

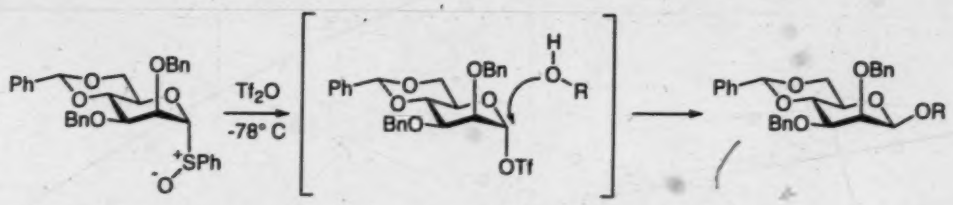


Figure 1.16 Use of mannosyl sulfoxides in the formation of β-mannosides

Other less general approaches have also been developed using dibutylstannylene complexes to activate the anomeric alcohol and to displace a leaving group on the accepting pyranose ring.[59] Investigation of quenching an anomeric radical from the axial direction has also been shown to be viable with primary alcohols.[60] Perhaps further developments may lead to improved yields and greater versatility in these approaches.

1.4 Enzymatic synthesis

The use of enzymes for carbohydrate synthesis has many advantages, the reactions are very efficient requiring no protecting group chemistry, no hazardous organic wastes are produced, and the enzymes are very specific limiting the necessity for difficult anomer and regioisomer separations.[61] There are three classes of enzymes that are commonly used in oligosaccharide chemistry, aldolases, glycosidases and glycosyl transferases.

The aldolases are used in the stereocontrolled formation of carbon-carbon bonds. These can be used to synthesize a variety of natural products and are usually only used by the

carbohydrate chemist when it is desired to introduce carbon-13 labels into specific positions in the pyranose ring.[62]

Glycosidases usually catalyse the hydrolysis of the glycosidic bond but this reaction can be run in reverse to form glycosides under conditions where an alcohol can effectively compete with water. This has been achieved using highly concentrated conditions, and by using activated glycosyl donors. Until recently these conditions have only lead to moderate yields of glycosides, but with cloning of the glycosidases and site directed mutagenesis, glycosidases have been developed that are unable to cleave glycosides but will synthesize them.[63] The active sites of these enzymes have been modified to remove the carboxylic acid necessary for catalysis of glycoside cleavage but, in the presence of an active glycosyl donor, glycosides can be formed. These enzymes have been termed glycosynthases.[64] Unfortunately product mixtures are often obtained due to the lack of specificity for the acceptor alcohol and thus these enzymes have found little use in the synthesis of complex oligosaccharides.

Glycosyl transferases are widely used in the synthesis of complex oligosaccharides. These enzymes transfer a monosaccharide moiety from nucleotide-activated donor sugars. They are very specific for the linkage formed but have been shown to tolerate many deviations from natural substrates, provided such deviations do not interfere with the key polar groups necessary for enzyme recognition.[65] Recently many of these enzymes have been cloned and are commercially available making them an obvious choice for the synthesis of oligosaccharides. Unfortunately enzymes are not yet available for all the linkages found in glycans. Furthermore, the nucleotide sugar donors are very expensive and remain difficult to synthesize.

The use of *in situ* co-factor regeneration alleviates the cost of nucleotide sugars in a synthesis by using a series of enzymes to recycle the nucleotide co-factors using an inexpensive high energy compound such as phosphoenolpyruvate.[66] Unfortunately, these recycling systems are difficult to set up and are only practical when a single oligosaccharide is desired on a large scale. Recently a more versatile approach using glycosyl fluorides as glycosyl donors for glycosyl

transferases has been investigated. It has been shown that these easily accessible compounds can be used as glycosyl donors by these enzymes, albeit at much reduced rate.[67]

As more of these enzymes are cloned and overexpressed, their use will no doubt become more popular among carbohydrate chemists. It is also reassuring to know that if a medically promising carbohydrate is discovered, investment in its enzymatic synthesis will make its production feasible on a large scale.

1.5 Conformational investigations of carbohydrates

Like polypeptides and polynucleotides, complex oligosaccharides adopt a range of three-dimensional conformations. Studies to determine the conformations of carbohydrates have revealed the binding mode of carbohydrates to proteins, which in turn has been used to design carbohydrate mimetics to inhibit certain binding events.[68] NMR analyses in conjunction with molecular modelling have been the primary tools used to obtain structural information about carbohydrates.

Unfortunately the use of X-ray crystallography is rare in the determination of carbohydrate conformations because of the challenges in crystallizing oligosaccharides. Only two trisaccharides, found in *N*-linked glycoproteins, have yielded X-ray quality crystals allowing their structures to be successfully solved. When this is compared to the number of protein structures that have been solved, the difficulty of this type of analysis for carbohydrates becomes apparent. More promising is the crystallization of carbohydrates with their binding proteins or as glycoproteins. This technique has given exceptional insight to carbohydrate protein interactions.[69] The use of NMR spectroscopy and molecular modelling in the determination of oligosaccharide conformation will be further elaborated in Chapter 5.

1.6 Scope of projects

Two oligosaccharides found to be involved in interesting biological events have been synthesized. These oligosaccharides both contain the synthetically challenging 1,2-cis-β glycosidic linkage and new methods for their synthesis were explored. The first oligosaccharide occurs as a unique antigen in a parasitic nematode, *Trichinella spiralis*. The oligosaccharide is hypothesised to be important in the parasite host interactions. The second oligosaccharide is found in the cell wall of the pathogenic yeast, *Candida albicans*. It is believed to be important in the host's immune response to this pathogen.

The *T. spiralis* glycan was synthesized to provide biological probes for the parasite host interaction. The oligosaccharide found in the yeast cell wall was studied in detail to determine its conformation and compare this conformation to a thioglycoside mimetic of the native structure. The solution conformation of this antigen was used to rationalise its immunochemistry and to design oligosaccharide neoglycoconjugates as vaccine candidates to immunize against *C. albicans*.

Chapter 2

Synthesis and biological investigation of *Trichinella spiralis* glycans

2.1 Introduction

2.1a Background

Trichinella spiralis is a parasitic nematode that is the causative agent of trichinosis. It has an exceptionally large host range and is endemic in many carnivorous animals. The parasite establishes itself in the intestinal epithelia of the host animal, following consumption of infected tissue and causes acute muscle pain, fatigue, fever, and in severe cases, death. James Paget first brought *T. spiralis* to the attention of science in 1835.[70] Continuing studies on this organism have revealed its life cycle, lead to measures to prevent infection and have discovered chemotherapies to combat infection by this nematode. *T. spiralis* infections have now been nearly eliminated from the first world through preventive measures such as meat inspection, sanitation and thorough cooking practices. Cases are still reported from the consumption of wild meat as is evident from the title of a recent publication in the International Journal of Infectious Disease,

"Trichinosis outbreak after ingestion of barbecued badger".[71] Benzimidazoles have emerged as a predominant chemotherapy and are effective if used early in the *T. spiralis* infection. Unfortunately *T. spiralis* infections are difficult to diagnose until the infection is well established, and in the absence of a sample of the tissue from the source of infection, only muscle biopsy or more recently immulogical methods can been used to identify a *T. spiralis* infection.[72] The large volume of literature surrounding this parasite makes it an attractive model for the study of parasite-host interactions.

The *T. spiralis* life cycle begins when a carnivorous host consumes infected meat allowing the digestion of tissue surrounding the parasite and the release of the infective larvae into the stomach. The larvae are then free to pass into the small intestine where they invade the columnar epithelial cells found in the intestinal wall. The larvae occupy over 100 epithelial cells in this stage of infection but do not destroy the cells in doing so. The mechanism of this invasion is not known although it is crucial to the initial parasitism of the host. Once established in this intramulticellular, stage the larvae undergo four molts in rapid succession to give the mature nematode. The mature nematodes then mate and, on approximately day 5 post infection, newborn larvae are passed into the lymph system. The newborn larvae move through the blood stream to sites of skeletal muscle where they invade, forming a new intracellular niche. Here the nematode grows and causes alterations in the striated muscle cells, allowing the formation of a nurse cell, where the larvae reside until this now infected tissue is consumed by a subsequent host.[70]

2.1b Importance of *T. spiralis* glycans

Rat pups suckling *T. spiralis* infected dams are found to acquire passive immunity, which eliminates 99% of an oral challenge of infective larvae.[73] This response is referred to as rapid expulsion, and is mediated by maternal antibodies that force larvae from their epithelial niche.

The antigens recognised by these protective antibodies are stage specific and are abundant in the excretory/secretory products (ES antigens) and cuticular surface of the infective larvae.[74]

Monoclonal antibodies have been isolated from rats infected with *T. spiralis* and these have been used to infer a functional role for glycoproteins in the pathogenicity of the parasite.[75] Eight clones have been isolated and were all of the IgG subclass of antibodies. Several of these antibodies have shown similar protective qualities to those passed to suckling rat pups from infected dams. Studies have shown that the antigens recognised by these antibodies are glycoproteins, of which the carbohydrate component is the immunodominant epitope.[76, 77]

Mass spectral analysis combined with enzymatic degradation was used to determine the structure of the *N*-linked immunodominant glycans. These have been identified as large tri and tetra-antennary structures, terminating in a rare 3,6-dideoxy-D-*arabino*-hexopyranosyl (tyvelose) linkage to a Lewis x like trisaccharide (Figure 2.1).[78,79] The triantennary structures isolated were truncated forms of the tetraantenary structure lacking either the 1-6 or 1-4 branching antennae. Tyvelose had previously only been observed in gram-negative bacterial lipopolysacharides.[80]

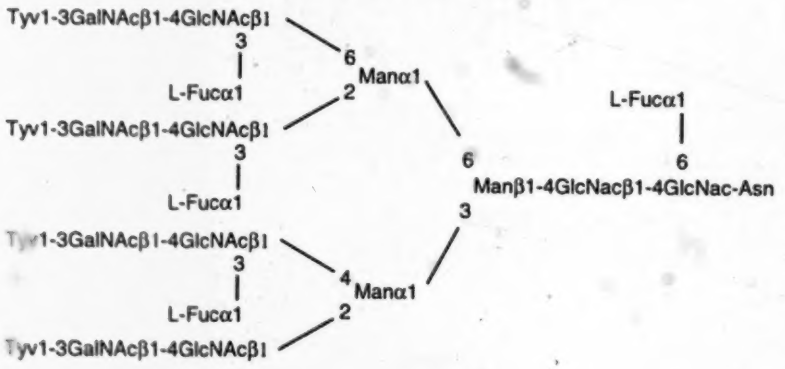


Figure 2.1. Tetra-antennary glycan found in excretory/secretory antigens of *T.spiralis*

Mass spectral analysis was not able to elucidate the anomeric configuration of the capping tyvelose residue due to the absence of a specific glycosidase for this linkage. In addition,

lack of adequate amounts of naturally occurring material precluded NMR studies. Thus, chemical synthesis of the terminal tetrasaccharide containing an α or β-linked tyvelose residue was undertaken so that immunochemical experiments could be used to infer the stereochemistry of the terminal linkage in the naturally occuring tetrasaccaride. It was confirmed through ELISA that the anti-*T. spiralis* antibodies bind the synthetic glycan when tyvelose is in a unique, β configuration.[81,82]

Inhibition of the binding of anti-*T. spiralis* antibodies to synthetic glycans established that not all the isolated monoclonal antibodies had the same fine structural specificity. They all bound structures containing a terminal β-D-*arabino*-hexopyranoside but some bound the synthesized tetrasaccharide (Figure 2.2) with high affinity, represented by monoclonal antibody 9D, and others bound the β-D-Tyv*p*-(1→3)-β-D-Gal*p*NAc-OMe disaccharide with high affinity, represented by monoclonal antibody 18H. Given that antibodies usually recognise three to six sugars of a glycan it was likely that the epitope for monoclonal antibody 18H contained tyvelose in the context of a different structure than found in the tetrasaccharide. The mass spectral analysis of the glycan chains had suggested that a non-fucosylated tyvelose capped structure might also be present on one of the antennae of the *N*-linked glycan (Figure 2.1 lower antennae). It was hypothesised that this non-fucosylated trisaccharide may in fact be the native ligand for the monoclonal antibody 18H (Figure 2.2).

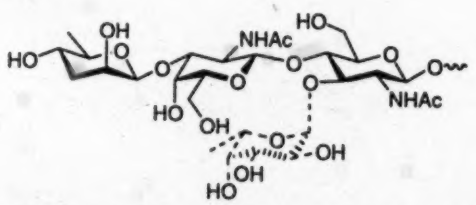


Figure 2.2. *T. spiralis* glycan capping tetrasaccharide. Dashed lines indicate partial fucosylation

2.1d Biological properties of *T. spiralis* glycan recognizing antibodies

Furthermore, the *in vivo* activity of the monoclonal antibodies 9D and 18H differ significantly.[83] *In vivo* 9D is protective against epithelial invasion of the parasite and 18H is only moderately protective, but *in vitro* 18H affords far greater levels of protection than 9D. Thus the small difference in fine structure between these antibodies seems to have large implications for the mechanism of the antibodies' action and consequently for the roles of the tyvelose capped epitopes these antibodies recognise.[84]

It has been observed that the monoclonal antibodies are able to form an immunoprecipitate around the anterior end of the parasite by crosslinking excretory/secretory antigens and antigens found on the surface of the larvae.[85] It was suggested that this immunoprecipitate prevented the parasite from receiving sensory information when exploring epithelial cells preventing invasion. Evaluation of the ability of monovalent antibody fragments, that are unable to crosslink antigens to form immunoprecipitates, to block invasion of epithial cells revealed that formation of an immunoprecipitate was not necessary to prevent invasion. However much higher concentrations of the monovalent antibody fragment were necessary to prevent invasion, suggesting the encumbrance of the immunoprecipitate may play a role in preventing parasite invasion, but this is not the only factor.[84]

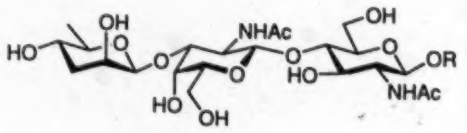
Recently, studies on the localisation of the ES antigens during invasion of cell cultures have shown a fraction to be localized to the nucleus of the epithelial cells under attack. This is interesting in light of another publication that has found single stranded endonuclease activity in the ES antigens.[86, 87] It would be interesting to explore the possible roles of the tyvelose containing glycans of this endonuclease.

At this point any hypothesis about a potential role of the tyvelose containing glycans is purely speculative. They may bind to the surface of the epithelial cells via C-type lectins that mediate a signal to the parasite as an initial step in pathogenesis. Only further study with defined glycans will allow the complex events necessary for parasitic invasion to be revealed.

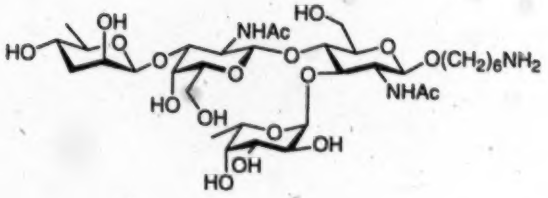
2.1e Scope of project

In order to investigate the fine structure of the monoclonal antibodies 18H and 9D raised during a *T. spiralis* infection it is necessary to have chemically defined ligands to screen against the antibodies. The synthesis of the methyl trisaccharide (**1**) will provide material to confirm the hypothesis that monoclonal antibody 18H recognizes this structure as its native ligand.

To investigate the potential roles for the surface glycans of this parasite in the pathogenesis of *T. spiralis*, chemically defined neoglycoconjugates are required that can be used to locate glycan receptors in tissue or cells. For this purpose epitopes labelled with a fluorescent reporter group were synthesised. This structure may be used in combination with fluorescence microscopy to probe for potential receptors for these glycans. Thus the trisaccharide was synthesized in a form amenable to the facile formation of fluorescent neoglycoconjugates namely compound **2**.



R=OMe **1**
=O(CH$_2$)$_6$NH$_2$ **2**



3

Figure 2.3 Target structures of *T.spiralis* glycans

Previous synthetic work involving this glycan included the synthesis of the terminal capping 3,6-dideoxy-D-*arabino*-hexopyranoside (tyvelose) as an anomeric mixture that was later

separated.[82] Recently developed techniques for the synthesis of this type of linkage in a diastereomerically pure form will be investigated. More classical approaches such as an oxidation and reduction approach at C-2 of the epimeric 3,6-dideoxy-D-*ribo*hexopyranoside (paratose) will also be investigated. It may then be possible to elaborate this structure enzymatically to the tetrasaccharide **3** containing the branching fucosyl residue using α(1→3) fucosyl transferase V. This would eliminate the difficulties previously encountered in chemical introduction of the 1→3 branching fucose, and provide access to the native ligand of monoclonal antibody 9D and potential neoglyconjugates of the tetrasaccharide.

2.2 Synthesis of *T. spiralis* glycans

2.2a Retrosynthetic analysis

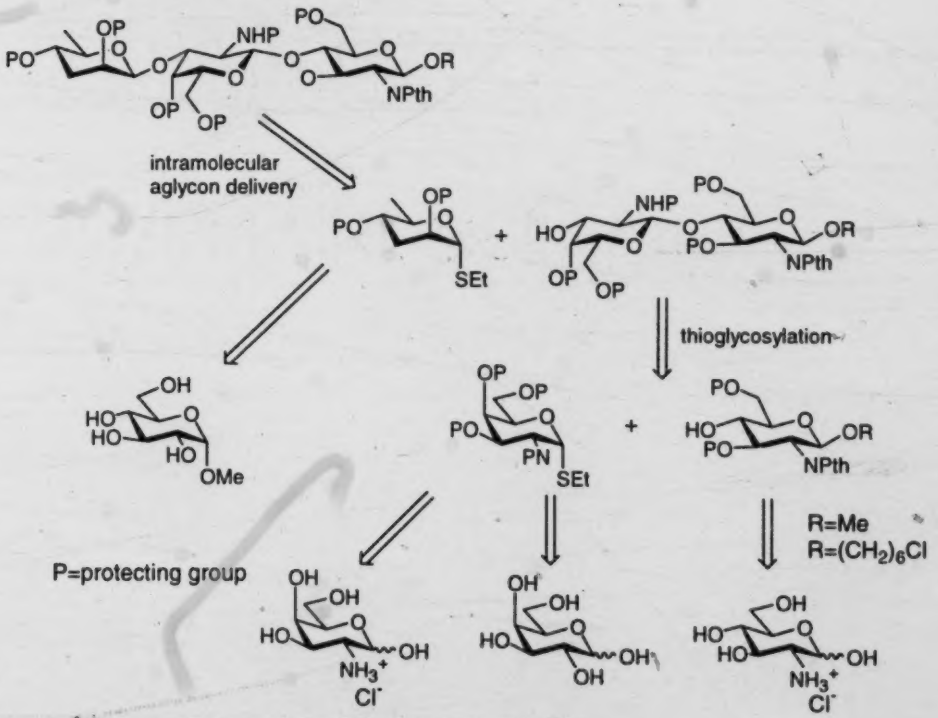


Figure 2.4. Retrosynthetic analysis of the target trisaccharide

The target trisaccharide contains three synthetic challenges that were addressed before beginning the synthesis: the manipulation of the amino protecting groups, synthesis of the 3,6-

dideoxy residue and, most challenging, the synthesis of the 3,6-dideoxy-β-D-*arabino*-hexopyranoside. It was decided to proceed from the reducing terminus in the synthesis, introducing the least readily accessible 3,6 dideoxy sugar last (Figure 2.4).

2.2b Amine protecting groups

Beginning at the reducing terminus, it was desirable to have a terminal amine separated by a short alkyl spacer as the aglycon of the trisaccharide in order to facilitate formation of neoglyconjugates of the final trisaccharide. This necessitates the manipulation of the three amino groups found in the trisaccharide in an orthogonal fashion. By employing a 1-chlorohexyl aglycon, a terminal azide could be introduced late in the synthesis after the protecting groups on the two amino sugars had been addressed. The azide would be orthogonal to acidic or basic conditions necessary for the deprotection of the other functional groups found in the trisaccharide.

Phthalimido protection was chosen as an ideal persistent protecting group for the glucosamine residue being stable to acidic, basic as well as a range of reducing conditions. It also minimizes many solubility and chromatographic problems which can be encountered with other amide and carbonate protecting groups.

Using commercially available galactosamine hydrochloride a variety of amide and carbonate protecting groups are accessible. It is also possible to start with galactose as an efficient method is available to introduce an azide at C-2 via the galactal. This flexibility is desirable to accommodate unforeseen problems integrating the amine protecting groups with the formation of the neighboring 3,6-dideoxy-β-D-*arabino*-hexopyranoside.

2.2c Synthesis of Tyvelose

The tyvelose glycosyl donor could be synthesized using an established synthesis in five steps from commercially available methyl α-D-glucopyranoside (Figure 2.5). This synthesis provides a selectively protected methyl α-D-tyveloside, which could be further manipulated to give the desired glycosyl donor.[88,89]

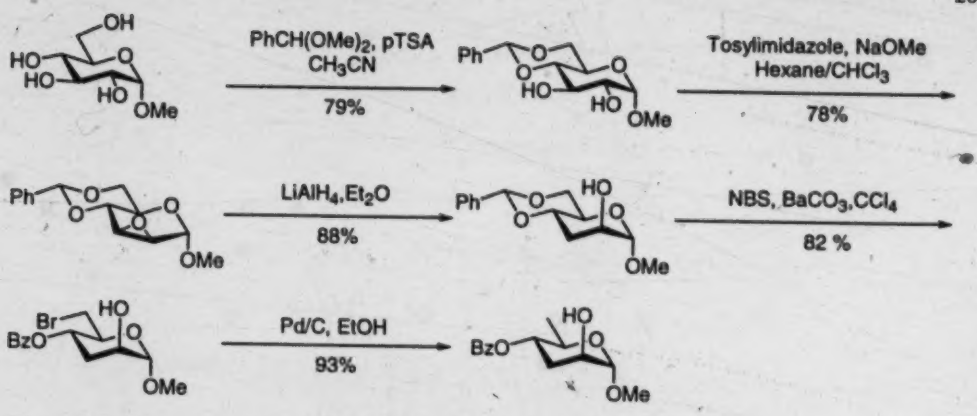


Figure 2.5. Literature synthesis of selectively protected methyl α-D-tyveloside

2.2d Formation of glycosidic linkages

The formation of glycosides is far from an exact science as the introductory chapter attests. In a retrosynthetic analysis one hopes to leave open as many different options for glycoside formation as possible. Thioglycosides were chosen as latent anomeric leaving groups because they have been successful for the formation of a variety of linkages in high yield, are easily synthesized, are stable to many manipulations, and can be easily interconverted to other anomeric leaving groups.

The galactosamine synthon was protected as a thioglycoside for formation of the LacdiNAc disaccharide. This disaccharide is of the 1,2-trans type outlined earlier and thus an acyl participating group at the 2 position of galactosamine is desirable.

At the time of these synthetic studies, three methods had recently been developed for the synthesis of β-mannosides based upon intramolecular aglycone delivery (see section 1.3a). These methods should be applicable to the formation of the 3,6-dideoxy-β-D-*arabino*-hexopyranoside. The first developed by Barresi and Hinsgaul utilized a 2,2-dimethyl acetal as the linkage between the glycosyl donor and acceptor.[39,40] This linker is more synthetically complicated than those developed later based on silyl acetals[43,44] and *p*-methoxybenzyl acetals.[46] These later methods were investigated for the synthesis of the 3,6-dideoxy-β-D-*arabino*-hexopyranoside.

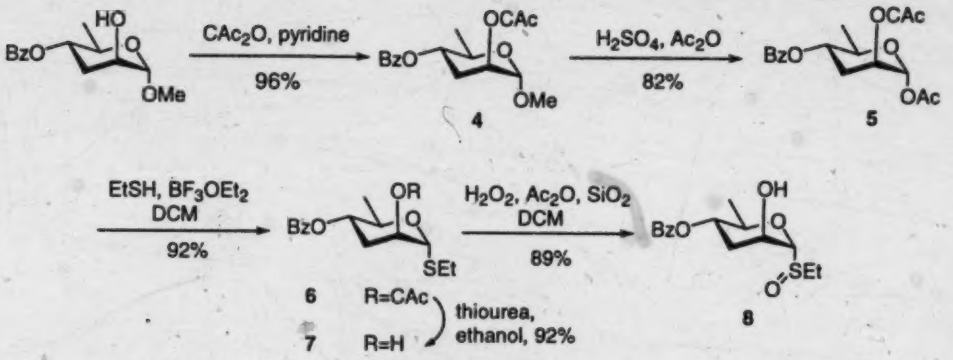
2.3 Synthetic studies toward the β-tyvelosyl linkage

2.3a The use of silyl acetals

A model study was undertaken to synthesize the 3,6-dideoxy-β-D-*arabino*-hexopyranosyl-(1→3)-β-D-galactoside to determine conditions that later could be applied to the synthesis of the trisaccharide. The first attempts using a silyl acetal as a tethering element required a stable glycosyl donor with a free hydroxyl at the 2 position and a selectively protected glycosyl acceptor.

The thioglycoside **7** was the first glycosyl donor investigated for this reaction (Scheme 2.1). Its synthesis began with methyl 4-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside available in five steps from methyl α-D-glucopyranoside.[90] This series of steps worked exceptionally well and was ameanable to large scales (10-15g).

The synthesis of **7** began with acylation of methyl 4-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside with chloroacetic anhydride to provide acyl protection at the 2 position, which was orthogonal to the benzoate already present in the structure to give compound **4**. Glycoside **4** was subjected to acetolysis conditions to give the anomeric acetate (**5**) necessary for generation of the thioglycoside (**6**) under Lewis acid promoted conditions with ethane thiol. The chloroacetate was then removed with thiourea in ethanol to give the selectively protected thioglycoside (**7**) in high yield. This compound could be further oxidized using hydrogen peroxide under heterogenous reaction conditions to give the glycosyl sulfoxide (**8**), the use of which will be explained later in the chapter.[91] These conditions for formation of the sulfoxide proved superior to the use of *m*-chloroperbenzoic acid in dichloromethane, which often leads to over oxidation to form the sulfone.



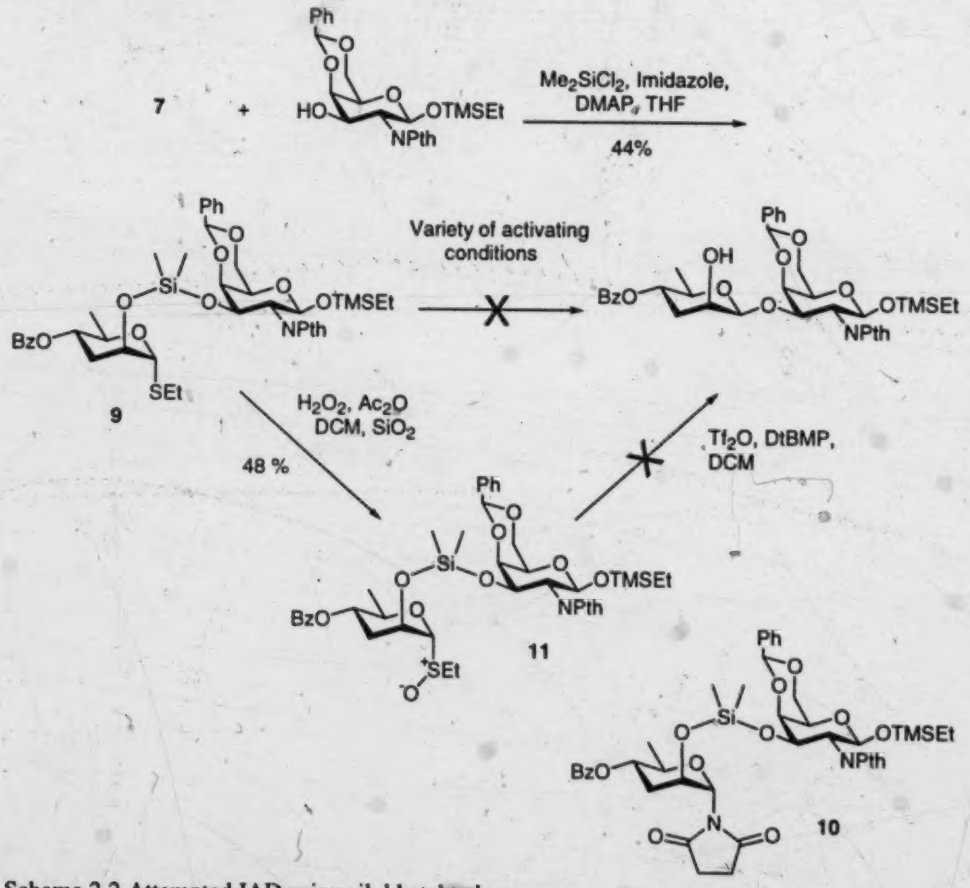
Scheme 2.1 Synthesis of selectively protected tyvelose donors.

The first selectively protected galactosamine derivative employed was the literature compound 2-(trimethylsilyl)-ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside, which was available in three steps from commercially available galactosamine hydrochloride.[92] The 2-(trimethylsilyl)-ethyl aglycon was chosen to permit manipulations at the reducing terminus for the exploration, if necessary, of synthesizing the trisaccharide from the non-reducing terminus.

Ethyl 3,6-dideoxy-1-thio-hexopyranoside **7** and 2-(trimethylsilyl)-ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside were linked to give **9** in low yield using a procedure published by Stork and La Clair (Scheme 2.2).[44] In this procedure the glycosyl donor and acceptor where stirred in an equimolar ratio with dichlorodimethyl silane and allowed to warm slowly. Provided the difference in activities are large between the two alcohols, no dimers should be formed. Unfortunately in this reaction dimers of both the 1-thio-tyveloside and the glycosyl acceptor were formed, attesting to similar reactivities between the alcohols of the donor and acceptor. If glycosylation had been successful, a sequential addition of the alcohols to prevent dimer formation may have optimized this reaction.[45]

The silyl acetal **9** was then subjected to a variety of activation conditions in hopes of collapsing the tether to the desired glycoside: NIS/AgOTf, NIS/TfOH, methyl triflate/di-*tert*-

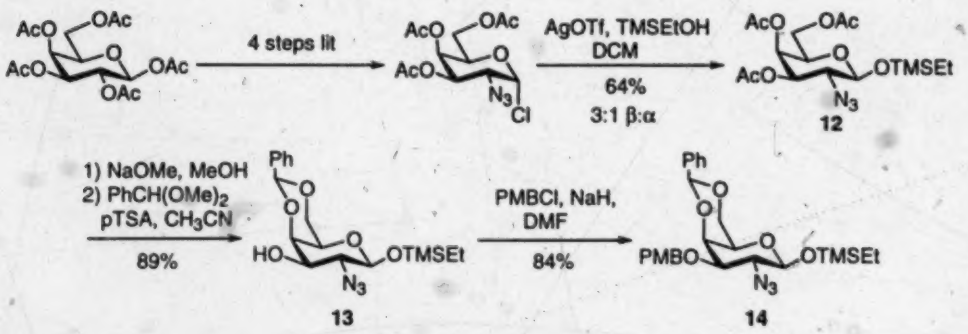
butylpyridine and iodine, all failed to yield an isolable amount of glycoside. The succinamide glycoside **10** was isolated when the reaction was run with NIS/AgOTf indicating the thioglycoside was being activated but the silicon tether failed to collapse. In an attempt to increase the activity of the glycosyl donor the thioglycoside was oxidized to the sulfoxide **11**. This was accomplished in moderate yield with a mixture of hydrogen peroxide and acetic anhydride in a slurry of silica gel. Upon activation of this compound a complex mixture was obtained from which only the glycosyl donor could be isolated and identified.



Scheme 2.2 Attempted IAD using silyl ketal tether

It has been well documented that glycosylation adjacent to a phthalimido protecting group can run into steric difficulties when glycosylation is attempted with a D-glycosyl donor

containing a participating group at the two position.[93] It was thought that steric factors might also be playing a role in the failure of the silyl acetal to collapse upon activation of the thiotyveloside (**9**). Thus a much less sterically demanding azide protecting group was investigated for formation of the disaccharide.

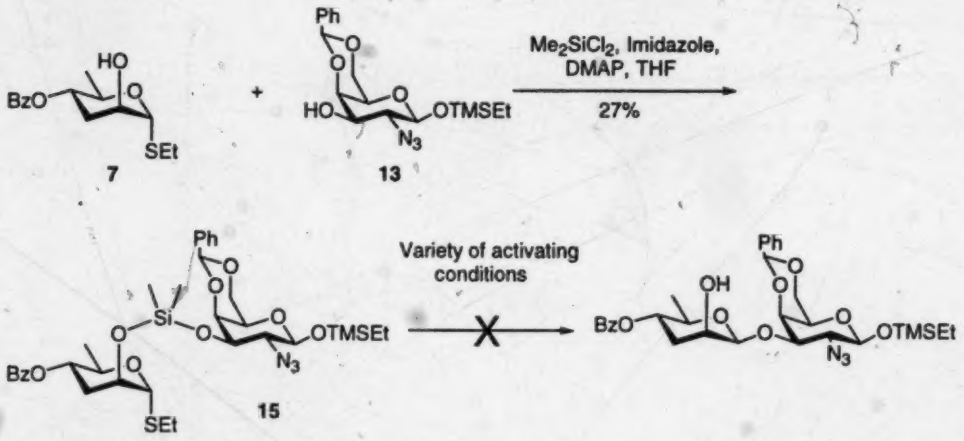


Scheme 2.3. Synthesis of 2-azido-2-deoxy-D-galactose glycosyl acceptors

The synthesis of this glycosyl acceptor began with galactose which, with a four step literature procedure via the 2,3,6 triacetyl galactal, furnished 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactosyl chloride (Scheme 2.3).[94] Activation of this glycosyl chloride in the presence of excess 2-(trimethylsilyl)-ethanol gave a 3:1 β:α mixture of the desired glycosides (**12**). The preponderance of β-glycoside in this reaction was surprising, given that azido glycosyl donors were developed to form α-glycosides. The conditions of the reaction and the reactivity of the acceptor alcohol obviously precluded the equilibration between the glycosidating intermediates necessary for α-glycoside selectivity (see section 1.2c). The anomers could be separated by silica gel chromatography and the β anomer was deacetylated and a 4,6-*O*-benzylidene was installed under standard conditions giving the desired glycosyl acceptor (**13**).

Unfortunately the donor-acceptor pair of **7** and **13** did not give any better results than had previously been achieved with the phthalimido protected acceptor (Scheme 2.4). The formation

of the silyl acetal (**15**) again was accomplished in low yield and dimers were isolated from the reaction. The silyl acetal failed to collapse under the same set of reaction conditions previously employed.



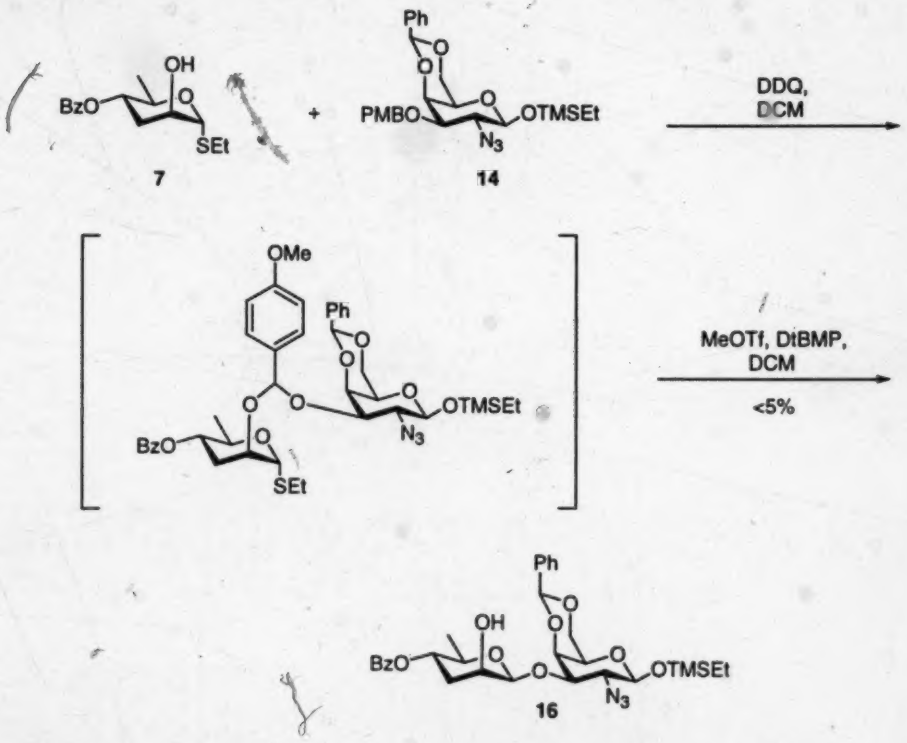
Scheme 2.4 Attempted glycosylations using a protected 2-deoxy-2-azido-galactosyl acceptor

2.3b Use of *p*-methoxybenzyl acetal tethers

Further efforts to form this difficult linkage intra-molecularly involved the *p*-methoxybenzidene acetal introduced by Ito and Ogawa.[46] The partially protected derivative **13** could be readily elaborated via alkylation to the desired *p*-methoxybenzyl functionalized glycosyl acceptor (**14**) necessary for attempting this glycosylation (Scheme 2.3). When this compound was oxidized by DDQ in the presence of glycosyl donor (**7**) and activated with methyl triflate a small amount of the desired glycoside (**16**) was isolated after chromatography (Scheme 2.5). No significant improvements in yield could be obtained by varying temperatures or activating conditions.

Different combinations of glycosyl donors and acceptors were investigated. Utilizing the glycosyl sulphoxide (**8**) and the *p*-methoxybenzyl derivatized acceptor (**14**) under similar reaction conditions resulted in a five fold increase in yield. But, after numerous variations of reaction

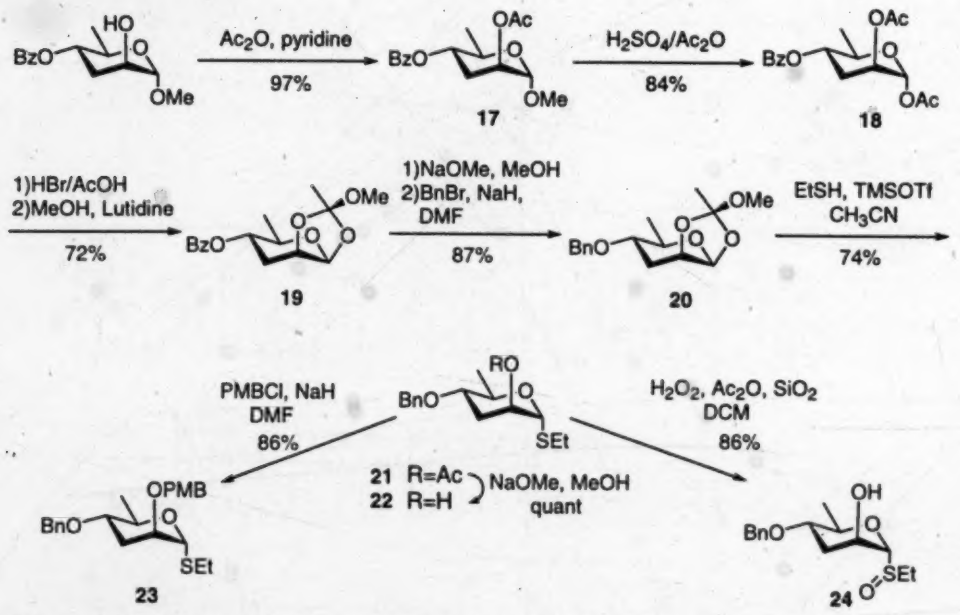
conditions, temperature, time, and purification only moderate further improvements were achieved (Scheme 2.8).



Scheme 2.5. Use of the *p*-methoxybenzyl acetal as a tether with thioglycoside donor **7**

Other combinations of glycosyl acceptor and donor were then investigated in hopes of finding a better combination. A more reactive glycosyl donor was investigated containing a benzyl protecting group at the four position, which has been shown to activate glycosyl donors when compared to acyl protecting groups.[95] This was synthesized beginning with the selectively protected methyl 4-*O*-benzoyl-2-*O*-3,6-dideoxy-α-*D*-*arabino*-hexopyranoside. Acetylation under standard conditions gave **17** which was subjected to acetolysis conditions to give the diacetyl derivative **18**. The exo-ortho ester (**19**) was then produced via the glycosyl bromide in two sequential steps in 72% yield. The ortho ester provided a convenient base stable protecting group which could be readily converted to a thioglycoside. The stereochemistry of the orthoester was

established by NOE investigations that showed close spatial relationships between the methyl group and H-4, and an interaction between the methoxy group and H-2. The benzoate could be removed with a solution of sodium methoxide and the benzyl group installed via standard alkylation conditions (**20**). The orthoester was then opened under acidic catalysis in the presence of ethanethiol to give the acetylated thioglycoside (**21**).[96] Deacylation gave the desired glycosyl donor (**22**) (Scheme 2.6).



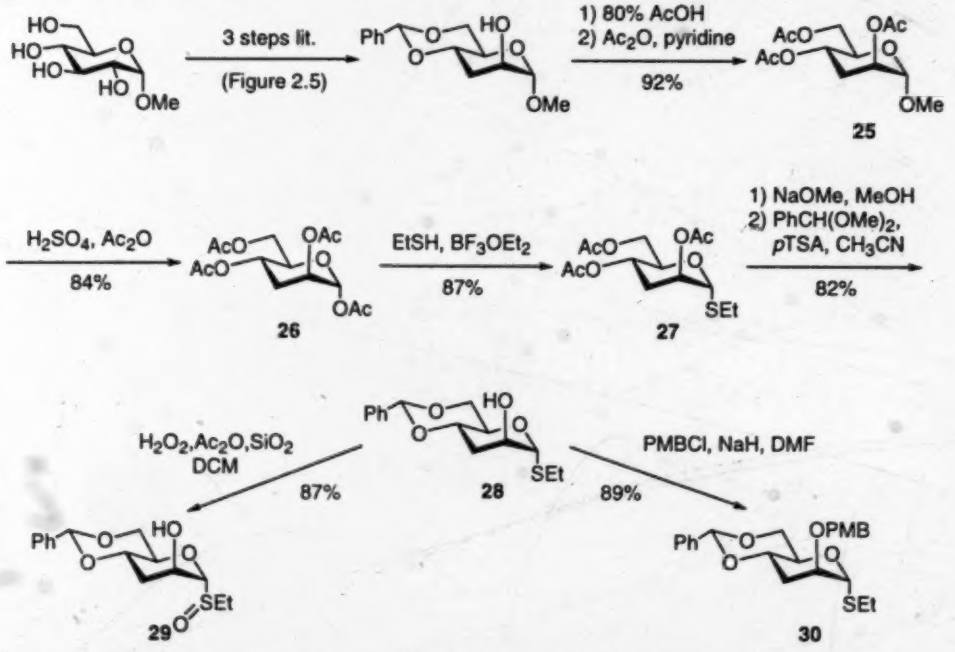
Scheme 2.6. Synthesis of 4-*O*-benzyl protected tyvelosyl donors

None of the desired glycoside was isolated when this donor (**22**) was combined with **14**, or when it was oxidized to the sulphoxide (**24**) and then condensed with **14**, under conditions that had previously led to the desired glycoside.

The reverse formation of the mixed *p*-methoxybenzyl acetal was also attempted by alkylating (**22**) with *p*-methoxybenzyl chloride to give glycosyl donor (**23**). When this donor was combined with accepting alcohol (**13**) none of the desired tyveloside was isolated (Scheme 2.8).

It was hypothesized that upon activation of the tyveloside, ring contraction may occur. This type of reaction has been observed for systems with good leaving groups at C-2 such as triflates and diazonium ions.[97] It has been observed that the presence of a 4,6-*O*-benzylidene ring prevents this type of reaction. Three possible donors were synthesized which contained a 4,6-*O*-benzylidene protecting group. It was thought that a selective bromination later in the synthesis would allow for introduction of the 6-deoxy functionality.[98]

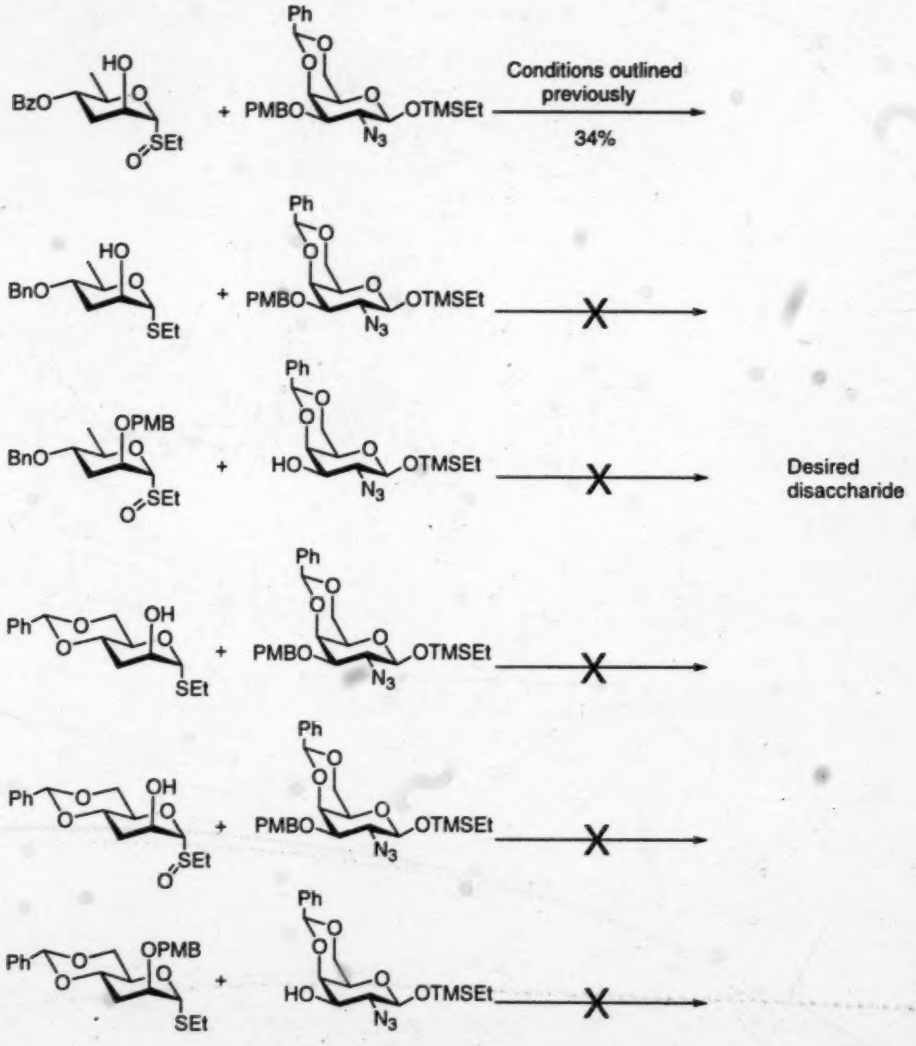
The synthesis of these donors started with a three step literature procedure from methyl α-D-glucopyranoside to give 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside (Figure 2.5).[88]



Scheme 2.7. Synthesis of 4,6-*O*-benzylidiene-3-deoxy-*ribo*-hexopyranosyl donors

The benzylidene ring was then removed by acid hydrolysis and the resulting triol was acetylated to give (**25**). Acetolysis of the triacetate (**25**) gave the 3-deoxy tetraacetate (**26**) which could be readily converted to the thioglycoside (**27**) under Lewis acid promoted conditions. The thioglycoside (**27**) was then deacetylated and the desired 4,6-*O*-benzylidene protecting group installed to give **28**. This compound could then be oxidized or alkylated in good yield to give a

series of glycosyl donors (**28**, **29**, **30**) fit for the intramolecular aglycon delivery reaction (Scheme 2.7). Unfortunately no desired glycoside was isolated from the combinations of **28** and **14**, **29** and **14** or **30** and **13** (Scheme 2.8). Only the glycosyl acceptor could be recovered, in moderate yield, from the reaction.



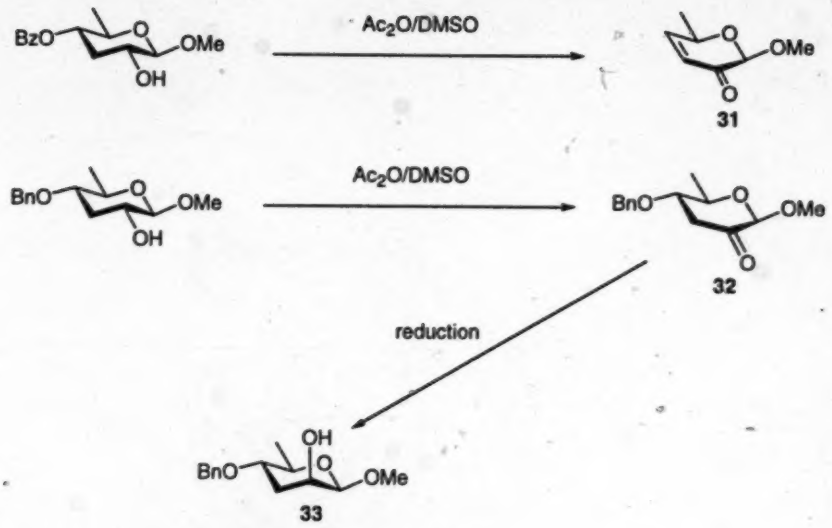
Scheme 2.8. Summary of combinations of glycosyl donor and acceptor used to in IAD with *p*-methoxybenzyl acetal tethers

It was decided at this point of the project that a more classical oxidation-reduction approach to synthesize this disaccharide might be more effective.

After the completion of this work a paper outlining the difficulties of intra-molecular aglycon delivery with p-methoxybenzyl tethers was published.[49] It described the importance of having a robust 4,6-*O*-benzylidene or better yet a 4,6-*O*-cyclohexylidene protecting group as well as a large protecting group at the 3 position. Given this information it is surprising that the 4,6-*O*-benzylidene protected donors (**28**, **29** and **30**) groups did not give better results. Evidently the lack of steric bulk at the 3 position of the tyvelose ring also dramatically affected this reaction.

2.3c Use of C-2 epimerization

The most general and robust method of synthesis of β-mannosyl linkages remains the oxidation and reduction of the C-2 alcohol to convert the 1,2-*trans* (2-D-glycero) to 1,2-*cis* (2-L-glycero) configuration.[99,100] The major challenge of applying this reaction would be the selective reduction of the uloside to the *arabino* glycoside. This was investigated using methyl 4-*O*-benzoyl-3,6-dideoxy-β-D-*ribo*-hexopyranoside previously synthesized in the Bundle laboratory. When this was oxidized under known reaction conditions, the result was a facile elimination and isolation of the β-D-glycero-hex-3-ene-2-ulo-pyranoside (**31**). After installation of a benzyl protecting group, this side reaction was eliminated giving a high yeild of the uloside (**33**) upon oxidation. Reduction was then attempted using a published procedure previously used to form a β-mannoside.[56] Using the suggested solvent and sodium borohydride a 1:1 mixture of epimers was obtained. When the more sterically demanding L-selectride was employed only the tyvelose epimer (**33**) was obtained. It was decided that this would be the method of choice to synthesize the β-tyveloside found in the target structures.



	Tyveloside	Paratoside
$NaBH_4$, EtOH/DCM	1	1
L-Selectide, THF	>98	<2

Scheme 2.9 Investigations of epimerization of paratose to tyvelose via oxidation reduction

2.4 Synthesis of the Tyvβ(1-3)GalNAcβ(1-4)GlcNAcβ trisaccharide by C-2 epimerization

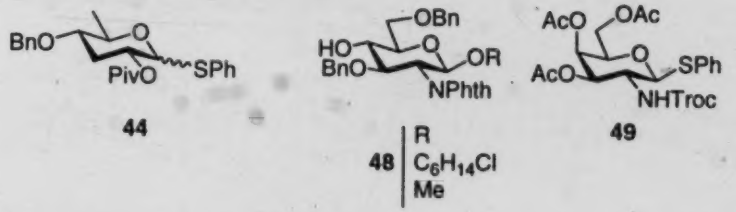
2.4a Retrosynthetic analysis

The success of the oxidation reduction approach to introduce the terminal tyveloside via its 3,6-dideoxy-D-*ribo*hexopyranose (paratose) epimer allowed a more rigorous analysis of synthons available to efficiently synthesize the target structures (**1,2**).

The thioglycoside donor (**44**) was synthesised with benzyl protection at C-4 and a participating pivaloyl ester at position C-2, ensuring β selectivity. This selectively protected donor also avoids complications of orthoester formation previously encountered for other paratose donors[101] and the elimination reaction previously observed upon oxidation. The synthesis of donor (**44**) took advantage of the *p*-methoxyphenyl group to protect the anomeric center throughout the series of manipulations required to produce the 3,6-dideoxy functionality.

This *p*-methoxyphenyl glycoside could be readily converted to the thioglycoside late in the synthesis. The use of this anomeric protecting group eliminated three steps in the synthesis that would have been necessary if the literature approach using the methyl glycoside was empolyed. It also illustrated the versatility of the recently developed *p*-methoxylphenyl anomeric protecting group.[102]

The glucosamine synthons **48** and methyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside[103] were employed as outlined above (section 2.2c). The galactosamine derivative **49** was chosen as an efficient glycosyl donor for the formation of LacdiNAc glycoside. It could be synthesized in high yield from the comparatively expensive galactosamine starting material. Furthermore, this choice of protecting groups provided three levels of orthogonal amine protection which helped to minimize solubility and chromatographic difficulties often encountered in small compounds with multiple acetamido groups.

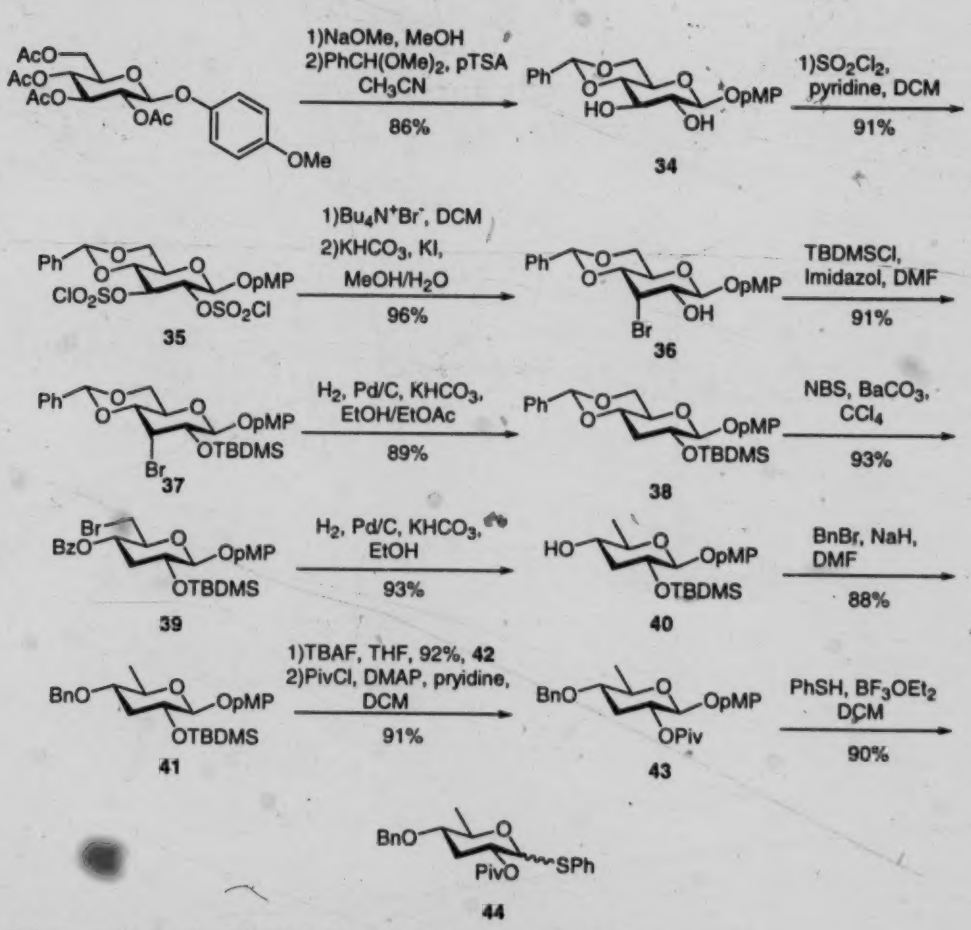


Scheme 2.10. Monosaccharide synthons

2.4b Synthesis of monosacharide synthons.

Beginning with the readily accessible *p*-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside,[102] deacetylation and conversion to the benzylidene acetal by treatment with benzaldehyde dimethyl acetal gave the highly crystalline compound **34**. Employing a series of high yielding steps introduced by Bundle,[104] the 3-deoxy-ribohexopyranoside (**43**) was synthesized. The crystalline bis-chlorosulphate ester (**35**) was cleanly prepared by treatment of the diol **34** with sulfuryl chloride. Reaction of the bis-chlorosulphate ester (**35**) with

tetrabutylammonium bromide followed by hydrolysis with an aqueous solution of potassium carbonate and potassium iodide gave the 3-bromo-3-deoxy-allopyranoside (**36**) in high yield. The stereochemistry at C3 was confirmed by proton NMR analysis of coupling constants, $^3J_{2,3} \approx {}^3J_{3,4}$ 3.4 Hz indicative of an axial orientation of the bromide.

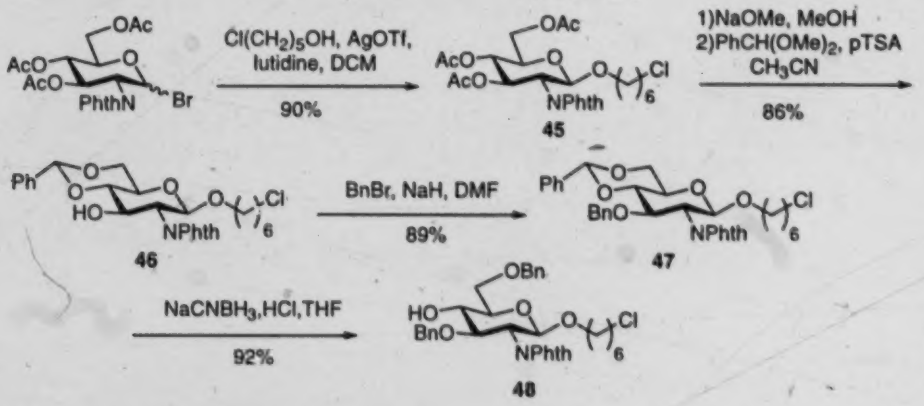


Scheme 2.11. Synthesis of 3,6-dideoxy-ribo-hexopyranosyl donor

Compound **36** was protected as the TDBMS ether (**37**) and hydrogenated over palladium to give the desired 3-deoxy-*ribo*-hexopyranoside (**38**). The 6-deoxy function was introduced in two steps via treatment of the benzylidene acetal with NBS to the give the 6-bromo derivative (**39**). The hydrogenolysis was accomplished in the presence of potassium carbonate in a solution

of ethanol resulting in concurrent debenzoylation to give the paratose derivative (**40**) in high yield. A benzyl ether was then introduced at C-4 under standard conditions (**41**). Removal of the silyl ether with tetrabutlyammonium flouride (**42**) and protection as the pivaloyl ester gave compound (**43**). Finally the *p*-methoxyphenyl glycoside could be readily converted to the thioglycoside by treatment with thiophenol and boron trifluoride diethyl etherate to give an anomeric mixture of the desired glycosyl donor (**44**) in 90% yield, confirming the viability of *p*-methoxyphenyl glycosides as useful intermediates (Scheme 2.11).

Methyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside was prepared according to literature procedures[103] and the 6-(chloro)hexyl glycoside (**48**) was prepared analogously (Scheme 2.12). The terminal chloride proved to be stable to the reductive opening of the benzylidene acetal and the basic Zemplen deacetylation conditions, providing a simple way to introduce a terminal azide later in the synthesis.



Scheme 2.12 Synthesis of glucosamine acceptors

2.4c Assembly of oligosaccharides

Galactosaminyl donor (**49**) was synthesized from the literature derivative 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranose by treatment with thiophenol and $BF_3{\cdot}OEt_2$.[105]

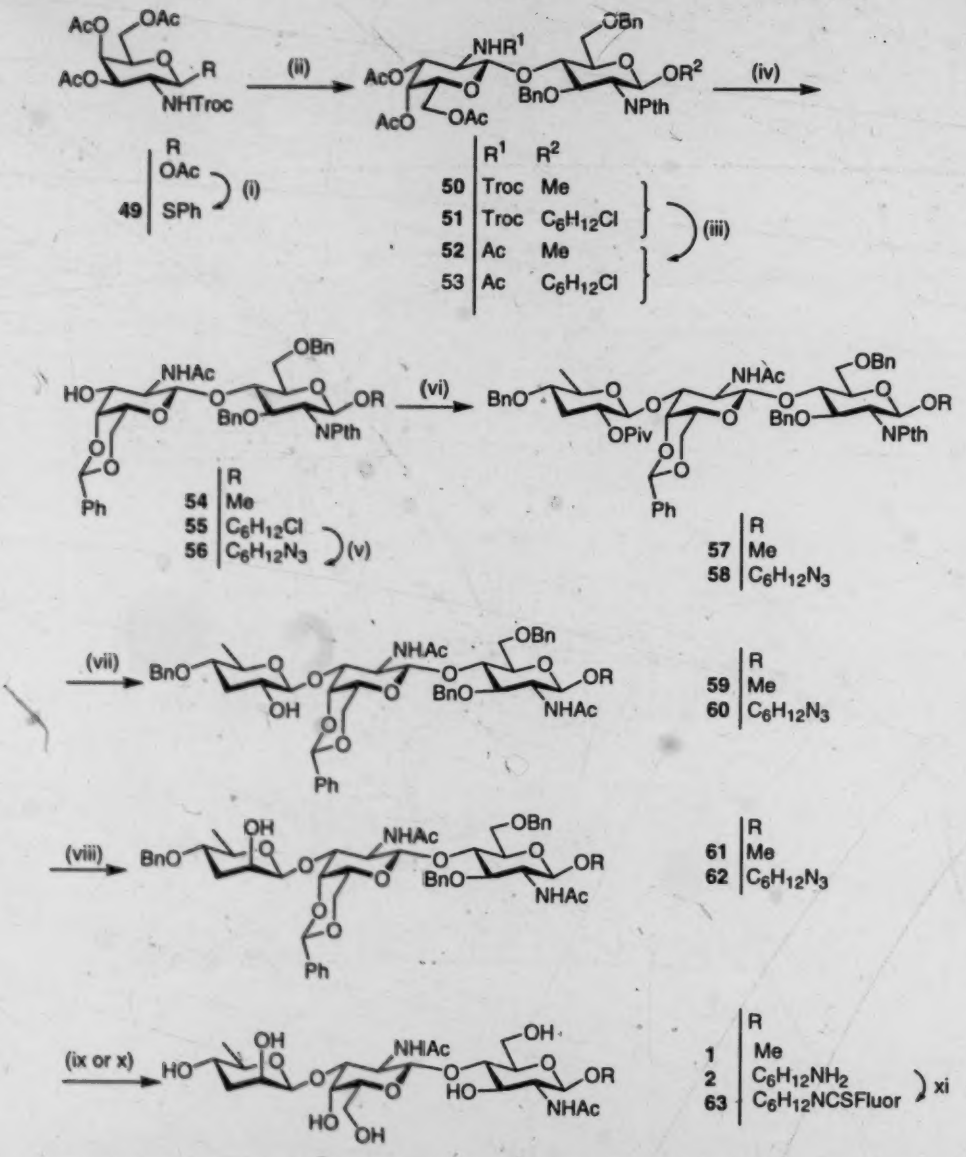


Figure 2.13. (i) PhSH, $BF_3{\cdot}OEt_2$ 74%; (ii) Acceptor, NIS, AgOTf, CH_2Cl_2, 87-93%; (iii) a) Cd, AcOH/DMF 1:1, b) MeOH, Ac_2O, 79-88%; (iv)a) MeONa/MeOH, b) $PhCH(OMe)_2$, *p*TSA, CH_3CN 79-85%; (v) NaN_3, DMSO, 96%; (vi) **44**, NIS, AgOTf, CH_2Cl_2, 81-85% (vii) a) $NH_2CH_2CH_2NH_2$, BuOH, b) Ac_2O, MeOH c) MeONa/MeOH, 85-88%; (viii) a) DMSO, Ac_2O, b) L-Selectride, THF 81% (ix) $Pd(OH)_2/C$, EtOH 88%; (x) $NH_2CH_2CH_2NH_2$, Li, 82%, (xi) 1:1 DMF/H_2O, $NaHCO_3$, FluorNCS 75%

Thioglycoside **49** proved to be an excellent glycosyl donor for the formation of glycosides **50** and **51** in greater than 87% yield using *N*-iodosuccinimide[106, 107] and a catalytic amount of silver triflate.[108] This combination of donor and acceptor gave higher yields than other combinations used to synthesize LacdiNAc.[105, 109] Cleavage of the trichloroethylcarbamate was most efficient and reproducible using cadmium metal in a 1:1 solution of DMF and acetic acid [110]. The free amine was acetylated in methanol to give the glycosides **52** and **53**. The benzylidene acetal was installed with benzaldehyde dimethyl acetal and toluenesulfonic acid after Zemplen deacetylation to give the target acceptors **54** and **55** in good yield. The terminal chloride was then easily displaced with sodium azide to give the orthogonally protected azide terminated linker (**56**).

Activation of paratose donor (**44**) with *N*-iodosuccinimide[106,107] and a catalytic amount of silver triflate[108] in the presence of the alcohol **54** or **56** gave the desired trisaccharides in **57** or **68** in greater than 75% yield. Unfortunately a 20:1 β:α ratio was obtained in the reaction which could not be separated by standard silica gel chromatography. However, the mixture was separated in subsequent steps of the synthesis. The pivaloyl and phthalimido protecting groups were removed in the next steps via treatment with ethylenediamine[111] at 110 °C for 16 h but these conditions failed to completely remove the pivaloyl ester. The free amine was acetylated by acetic anhydride in methanol, and the remaining pivaloyl ester was removed with a solution of sodium methoxide in methanol to give the glycosides **59** and **60**.

The oxidation and reduction[99,100] of the paratose glycosides **59** and **60** was accomplished in two steps performed sequentially. Treatment for 18 h with a 2:1 dimethyl sulfoxide and acetic anhydride mixture, followed by removal of the volatile components under high vacuum, gave the corresponding ulosides as yellow solids. It was important to use freshly distilled DMSO as this inhibited the formation of the methylthiomethyl ether often encountered under these conditions.[112] Reduction with excess L-selectride gave the tyvelose terminated trisaccharides (**61**,

62). Quenching the reaction of the 6-(azido)hexyl glycoside (**60**), with ethylenediamine avoided reduction of the azide and gave **62** in 75% yield.

The inversion of stereochemistry at C-2 was confirmed by NMR studies. The $^{3}J_{1,2}$ value <1Hz was indicative of a 1,2-*cis*-mannosyl linkage,[113] while the heteronuclear $^{1}J_{C1,H1}$ constant of 158 Hz unambiguously established the β configuration.[114] No products with the *ribo* configuration were observed in the NMR spectrum of the crude product, indicating that the selectivity of the reduction was better than 95%.

Final deprotection of **61** and **62** gave the desired trisaccharides **1** and **2**. The methyl glycoside was hydrogenated over palladium hydroxide and could be crystallised from methanol/ethanol mixtures as fine needles in 93% yield. The 6-azidohexyl trisaccharide was deprotected under dissolving metal conditions[115] using ethylenediamine as the solvent due to low solubility of the LacdiNAc structure in THF-ammonia mixtures. Final purification by HPLC and lyophilization gave **2** as a white powder in 82% yield. The 6-(amino)-hexyl tether was then simply derivatised as its fluoresceine conjugate by stirring with fluoresceine isothiocynate in a DMF-water solution, followed by purification to give **63** in 75% yield.

2.4d Enzymatic elongation reaction

With an efficient synthesis of the linear trisaccharide epitope accomplished, it was decided to elaborate the structure enzymatically to the tetrasaccharide epitope, as these structures would also be helpful in the biological studies of the glycan.

In oligosaccharide synthesis glycosyl transferases have found extensive application due to their ease of use and recent commercial availability. The terminal tetrasaccharide (**3**) under consideration is related to the Sialyl Lewis^{x} epitope found in many glycans. It was envisaged that the α(1→3)fucosyl transferase V, known to synthesize this glycan, would recognize the linear trisaccharide (**2**). There are several differences between the two structures. Sialic acid is replaced

by tyvelose, which should be tolerated given the finding that the LacNAc disaccharide is accepted by the enzyme.[116] Furthermore the 2-OH of the galactose residue on the natural structure has been replaced by an acetamido group. The enzyme would also likely tolerate this modification as the enzyme has been shown to fucosylate LacdiNAc (Figure 2.10).[117]

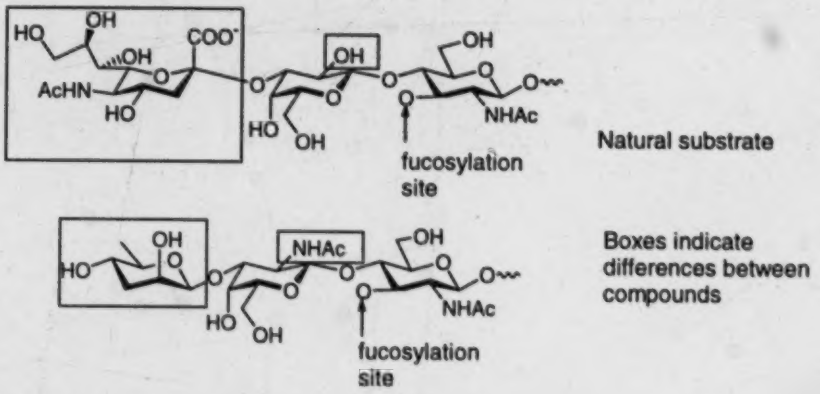


Figure 2.10. Comparison of acceptors for α-(1-3)-fucosyl transferase V

The fucosyl transferase reaction worked very well on 5 mg scales using the aminohexyl tether trisaccharide **2**. Initial kinetic investigations showed the transferase reaction to be at least as rapid as with octyl β-lactosamine. The product could be easily isolated by reverse phase chromatography to give the desired product (**3**) in 74% yield.

2.5 Preliminary biological results

The two monoclonal antibodies were assayed to determine the affinity of the linear trisaccharide relative to the tetrasacchride. An ELISA inhibition assay developed in the Bundle laboratory was employed for this task.

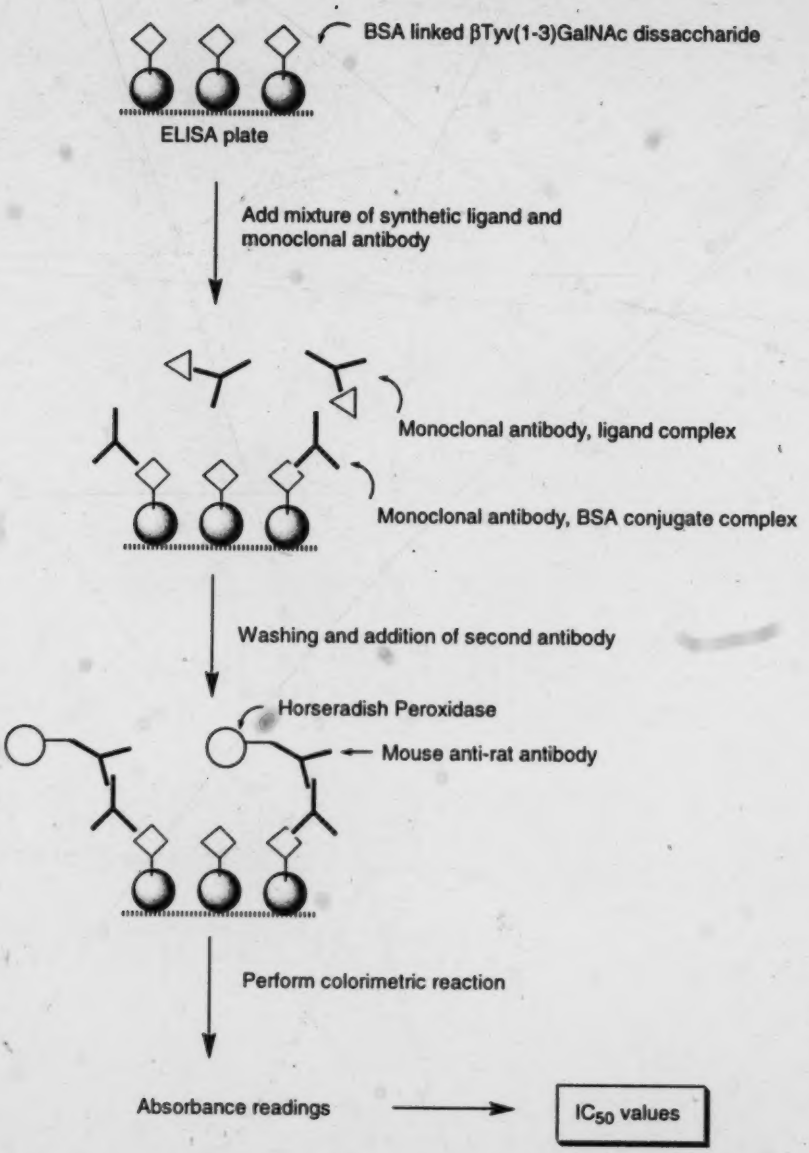
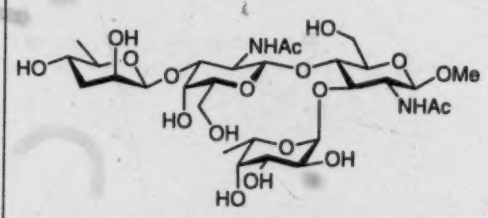
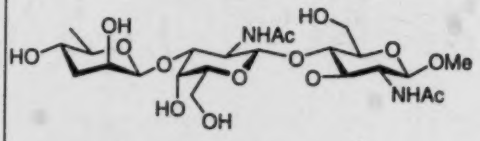
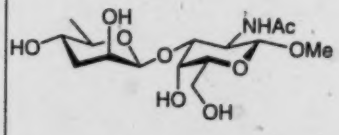


Figure 2.11 ELISA used to quantify ligand binding to anti-*T. spiralis* antibodies

In this assay βTyv(1→3)βGalNAc disaccharide covalently linked to bovine serum albumin, which had been previously synthesised,[82] was coated on the ELISA plate. A solution containing the antibody of interest and the ligand being investigated was then added to the plate. The ligand then competes with the disaccharide bound to the plate for the antibody. After

washing away unbound reagents, the antibody bound to the antigen coated plate is quantified. This is accomplished by adding a second antibody that recognizes the first antibody and has a covalently attached enzyme which is capable of catalyzing a colorimetric reaction. In this case the second antibody is linked to horseradish peroxidase which is capable of oxidizing tetramethyl benzil to a coloured product (Figure 2.11). The results from this assay are shown as percentage inhibition and the concentration of ligand that gives 50% inhibition is quoted as an IC_{50} value. The lower this value, the higher the affinity of the ligand for the antibody being studied.

Table 2.1. ELISA inhibition results

Ligand	Antibody (IC_{50}) 9D	18H
	15μM	>500 μM
	58 μM	~480 μM
	178 μM	93 μM

Two monoclonal antibodies were assayed under these conditions. The first, 9D, is known to bind the tetrasaccharide strongly and have a weaker affinity for the disaccharide. The second, 18H, is known to bind the tetrasaccharide weakly but the disaccharide strongly (Table 2.1).

Antibody 9D had the expected affinity for the trisaccharide, falling between the disaccharide and the tetrasaccharide, having 3 times higher affinity than the disaccharide, and five times weaker affinity than the tetrasaccharide. This indicates the binding site of this antibody

does have significant contacts to all four sugars of the tetrasaccharide, while the majority of its binding energy is focused around the dideoxyhexose, an observation that has been found for other antibodies recognizing glycans containing dideoxyhexoses.

Antibody 18H unexpectly recognized the trisacharide with an affinity comparable to that of the tetrasaccharide and recognized the disaccharide with 6 times higher affinity. It had been hypothesized that this linear trisaccharide structure would be bound more tightly than the branched tetrasaccharide indicating that the (18H) monoclonal antibody had been raised against the non-fucosylated linear arm of the glycan. Evidently, this is not the case.

This raises the question as to what native epitope induced the formation of monoclonal antibody 18H. Other glycans that bare capping tyvelose residues must be present in *T. spiralis* that contain the natural epitope for 18H. In the process of isolating the glycan sequenced in mass spectral analysis, an affinity column made up of the 9D monoclonal antibody was used to fractionate the excetory/secretory anitigens, possibly eliminating those which bind to 18H. Furthermore, the isolated proteins were then treated with PNGase F, an enzyme which specifically removes *N*-linked glycans. Thus, any O-linked glycans with other structures would have been lost in this purification process. It has also been found that total larval homogenates and antigens isolated from a polyclonal affinity column have significantly different monosaccharide compositions when analyzed by GC/MS than the excretory/secretory antigens used for the glycan structural determination.[118] To determine the native epitope of 18H it is necessary to go back and repeat the process with an affinity column made up of this antibody to see if other tyvelose capped glycans can be isolated. It may also be necessary to isolate the glycans at different phases of infection, as it is likely that glycan structure is dependent on the life cycle of the nematode.

Investigations into potential C-type lectins which bind the tyvelose capped antigens are ongoing in Judith Appleton's laboratory at Cornell University.

Chapter 3

Synthesis of (1→2)-β-D-mannopyranan oligomers found in the phosphomannan of *Candida albicans*

3.1 Introduction

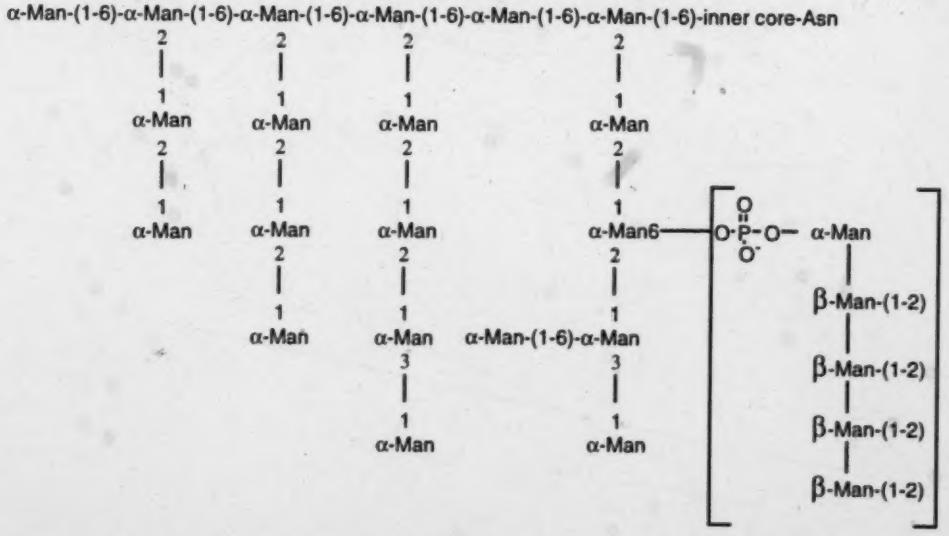
3.1a Background

Although *Candida albicans* is ubiquitous in the natural flora of humans it is also the most common etiologic agent in candidiasis.[119] This infection commonly occurs in immunocompromised patients and those undergoing long term antibiotic treatment.[120] The infection can range in severity from a common mucosal infection to a life threatening systemic infection. Regular increases in the number of cases of systemic candidiasis have become a major medical problem in hospitals where *C. albicans* is now responsible for up to 25% of the nosocomial infections.[120] This increase is associated with increasing drug resistance to available anti-fungals and the difficulty with early detection of *C. albicans* infections.[121] Considerable effort is now being expended to understand the interaction between the host and this fungal pathogen in hopes of finding new treatments for this troublesome organism. It appears now that there are many subtleties in the host-pathogen interplay, reminiscent of two well-antiquated foes adapted to one another's tactics.

3.1b Candida albicans cell wall

The first line of defense for *C.albicans* is its cell wall, consisting of mainly chitin, glucan and mannan. The chitin and glucan are protective against osmotic stress allowing the yeast to live in a wide range of environments, inside and outside the host. The mannan portion of the cell wall has received the greatest attention as it is highly immunogenic[122] and has been associated with the adhesion of yeast to different cell types.[123,124] It is known to cause activation of macrophages[125], and is the mechanism whereby yeast bind to macrophage membranes.[126,127]

Suzuki *et al.* using NMR and mass spectrometry have elucidated the structure of the mannan component of *Candida albicans*.[128] Less than 10% of the mannan is O-linked, consisting largely of short (1→2)-α and (1→3)-α-D-mannopyranan.[129] The complex *N*-linked components are composed of extended (1→6)-α-D-mannopyranan backbones containing (1→2)-α-D-mannopyranan branches. Furthermore (1→2)-β-mannopyranan oligomers are attached through a phosphodiester bridge. The position of attachment of this phosphodiester has yet to be determined (Figure 3.1). The mannan is heterogeneous and differences in mannan chain length have been shown to be nutrient and environment dependent.[130]


α-Man-(1-6)-α-Man-(1-6)-α-Man-(1-6)-α-Man-(1-6)-α-Man-(1-6)-α-Man-(1-6)-inner core-Asn
α-Man
α-Man
α-Man

Recently the mannan has also been shown to exist as phospholipomannan.[131] This is a new type of eukaryotic glycolipid characterized by the absence of *N*-acetylglucosamine. The glycolipid contains an eight to eighteen residue (1→2)-β-mannan oligomer that is linked via phosphoinositol to a yet to be identified lipid.

3.1c Host defenses against fungal infection

Work is continuing on the host defenses against *Candida albicans*, and it appears that both humoural and cell mediated immunity play major roles. The most serious cases of fungal infections occur in patients with defects in their cellular immunity, but the role of antibodies also appears to be crucial in these infections.[132] The patients with recurring candidiasis seem to lack antibodies against a large portion of the mannan found in the yeast cell wall.[133]

Monoclonal antibodies raised against the *C. albicans* cell wall extracts in mice were protective against disseminated candidiasis and vaginal candidiasis.[134,135,136] Further studies on these monoclonal antibodies indicated the active epitope to be a portion of the (1→2)-β-mannan polymer found in the phosphomannan.[137] Agglutination studies suggested the epitope was likely to be a (1→2)-β-mannotriose derivative. This is a curious result as the authors found longer (1→2)-β-mannooligomers were not able to prevent agglutination of antibody coated latex beads and Suzuki *et al.*[186] found that longer oligomers predominate on the surface of *Candida albicans*.

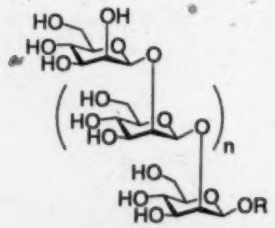
The role of cell mediated immunity in mucosal infections is complex. Antibodies have been shown to be protective, but local impairment of cellular immunity to *C. albicans* appears ubiquitous in vaginal infections. The possibility that the mannan is responsible for down regulation of the cell mediated response through a T-cell derived antigen binding molecule is a possibility that is being explored.[138] *C. albicans* also sheds a large amount of phospholipomannan when in the presence of macrophages, causing intense secretory and signaling responses in these cells.[139] This is interesting given the recent finding that a population of T-cells is able to process

glycolipid antigens analogously to peptide antigens via MHC-like Cd1d molecules.[140] It is tempting to speculate that the phospholipomannan may be playing a role in the manipulation of the immune response to facilitate *C. albicans* infections.

Attempts to isolate and clone the mannosyl transferase responsible for the synthesis of (1→2)-β-mannosyl oligomers have been troublesome. A β-mannosyl transferase has been cloned but this enzyme is not able to elongate the (1→2)-β-mannosyl polymer.[141] It is likely that continued efforts will find the productive enzyme, making the efficient synthesis of these oligomers possible. However, until that time, studies dependent on chemically defined antigens will rely on their isolation or chemical synthesis.

3.1d Scope of project

The importance of having well defined systems to elucidate the role of the (1→2)-β-D-mannopyranan in *C. albicans* infections and to investigate the potential of this structure for developing effective vaccines against *C. albicans* can most easily be realized through the synthesis of these oligosaccharides. The chemical synthesis of a (1→2)-β-D-mannopyranosyl hexamer and its lower congeners was developed in a flexible fashion to allow for facile synthesis of glycoconjugates or the synthesis of simple glycosides (Figure 3.2).



R= functionality to facilitate glycoconjugate formation or simple alkyl glycoside
n=1-4

Figure 3.2. Synthetic targets. Derivatives of structures found in the phosphomannan of *C. albicans*

This synthesis involved developing an efficient strategy for the formation of contiguous β(1→2) mannoside linkages. The aglycon will be manipulated to generate a terminal amine that can be coupled to form glycoconjugates or transformed to a simple short alkyl sequence.

3.2 Synthesis of (1→2)-β-D-mannopyranosyl oligomers

3.2a Retrosynthetic analysis

Flexibility in the aglycon was desired in the final steps of the synthesis for the formation of functionality necessary for glycoconjugate formation or to produce simple glycosides. Numerous methods for glycoconjugate formation are available but the most facile and efficient rely upon coupling through a free amine. The allyl group served as an ideal surrogate for this functionality, providing a robust persistent anomeric protecting group which could be transformed into a variety of functionalities via photo addition of a thiol late in the synthesis, or oxidation to the aldehyde followed by reductive amination.[142] Allyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside was targeted as a desirable building block for the reducing terminus of the oligomers. The allyl β-D-mannopyranoside could be synthesized from the glucoside via an oxidation-reduction approach that is feasible on the large scale necessary at this early stage of the synthesis (Figure 3.3).

The rational synthesis of β-mannopyranosides is a longstanding problem in glycoside synthesis, which lacks a general solution, despite several novel approaches (outlined in chapter 1). All of the general methods developed for formation of the β-mannosides rely upon manipulation at C-2 of the non-reducing mannoside, via oxidation/reduction or tether attachment for IAD. In the case of (1→2)-β-D-mannopyranan oligomers this necessitates a linear synthesis from the reducing terminus. The formation of the β-mannosides began by investigation of the use of methods known to give exclusively the β-mannopyranosyl linkage, since difficulties were anticipated in the purification of these homopolymers. We were understandably reluctant to investigate IAD in this case, given the difficulties encountered in the synthesis of the *T. spiralis* glycan, and began investigations using a ulosyl bromide donor developed by Litchenthaler *et al.*[55,56] In the elaboration of a 1→2 linked polymer by this approach the uloside is obtained and this product can be reduced to afford directly the selectively protected glycosyl acceptor required

for the next glycosylation step. This was a desirable approach because of the reported high diastereoselectivity over both the glycosylation and reduction steps, and the minimization of the number of protecting groups required. Furthermore, the known 3,4,6-tri-*O*-benzyl-1,2-*O*-(exo-ethoxyethylidene)-α-D-glucopyranose intermediate used in the synthesis of the reducing terminus serves as a direct precursor for the synthesis of the ulosyl bromide lending efficiency to the synthesis.

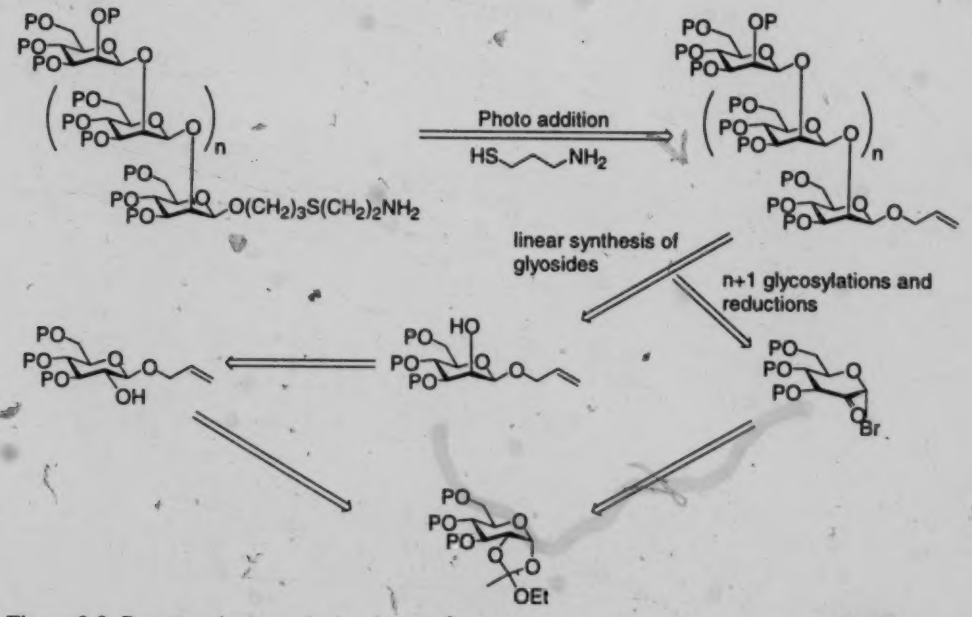
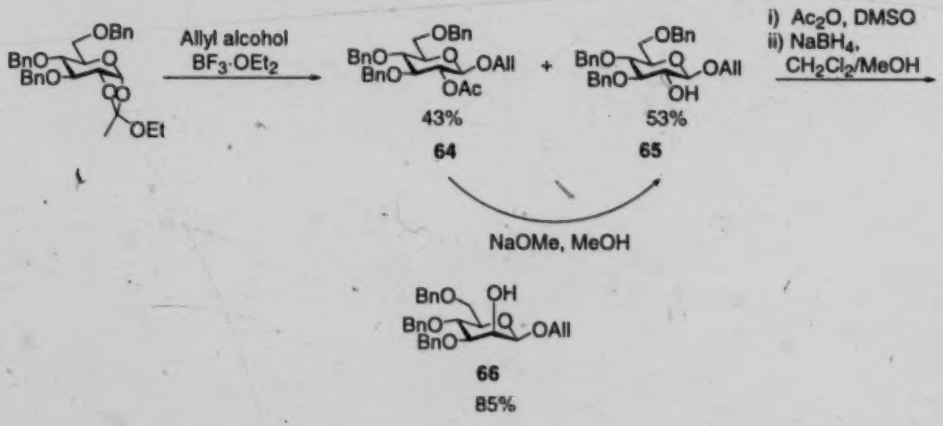


Figure 3.3. Retrosynthetic analysis of (1-2)-β-mannopyranosides

Benzyl protecting groups were chosen to protect the hydroxyl functionality not participating in the synthesis. These could be easily installed around the orthoester providing the required selectively protected intermediates, that would be and were stable to the reducing conditions used throughout the synthesis. Finally the protecting groups could be removed under catalytic reduction or dissolving metal conditions, which may be necessary to avoid potential catalyst poisoning by a thioether, that would be introduced into the aglycon (Figure 3.3).

3.2b Synthesis of reducing terminus

Allyl 3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (**66**) could be conveniently synthesized on a large scale (up to 10g) starting from 3,4,6-tri-*O*-benzyl-1,2-*O*-(exo-ethoxyethylidene)-α-D-glucopyranose.[143] Lewis acid promoted glycosylation in allyl alcohol gave a high yield of a near 1:1 mixture of the expected product **64** and its deacetylated counterpart **65**. The allyl-β-D-glucopyranoside **64** was treated under Zemplén deacylation conditions to give the desired crystalline alcohol **65**. Oxidation of the glucopyranoside (**65**) using acetic anhydride and DMSO and reduction with sodium borohydride gave the mannopyranoside (**66**) in good yield with high selectivity (Scheme 3.1).[144] Using freshly distilled DMSO and anhydrous conditions minimized the commonly encountered methylthiomethylether side product, as previously outlined.[144]



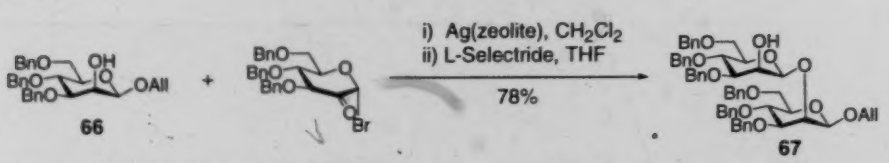
Scheme 3.1. Sythesis of reducing terminal allyl glycoside

3.2c Synthesis of glycosides

The synthesis of the first 1,2-linked β-mannopyranosyl unit was accomplished using conditions similar to those employed by Lichtenthaler *et al.*[55] The glycosyl donor 3,4,6-tri-*O*-benzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide was synthesized via the literature route (see Figure 3.4 as an example of this route), although the yields for the thermal rearrangement of the

orthoester (benzyl analogue of **70**) into an acetoxyglycal (cf. **71**) were approximately 65% compared to 85% in the literature.

Using silver exchanged zeolite[145] to promote the glycosidation of **66** by 3,4,6-tri-*O*-benzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide, followed by reduction of the product with L-Selectride gave the desired disaccharide (**67**) in excellent yield after purification (Scheme 3.2). No α-*manno* anomer or *gluco* epimers were isolated from the reaction mixture. It was necessary to use the sterically hindered reducing agent L-Selectride for this reduction since in contrast to the monosaccharide, the disaccharide gave epimeric mixtures when sodium borohydride was employed.

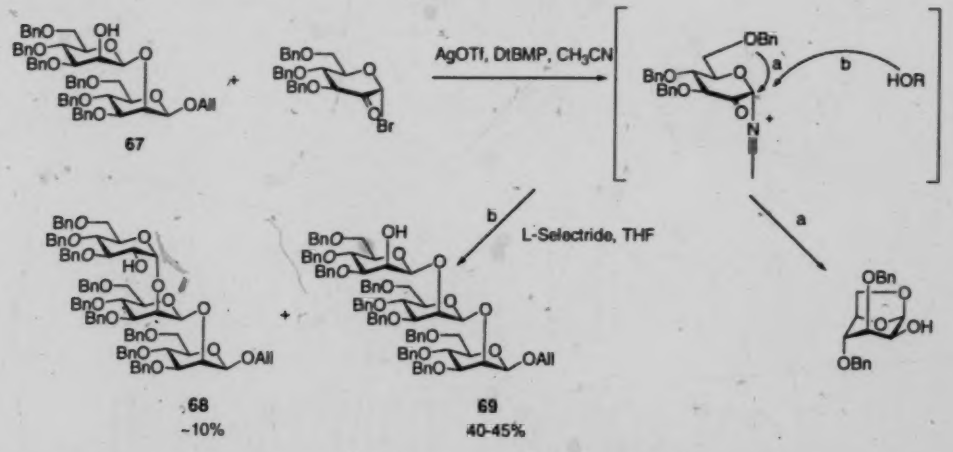


Scheme 3.2. Synthesis of allyl β-D-mannopyranosyl-(1-2)-β-D-mannopyranoside

Introducing subsequent β-mannopyranosyl units proved more difficult and the conditions employed for disaccharide synthesis failed to yield significant amounts of trisaccharide. Exploration of different activation protocols led to the use of the soluble promoter, silver triflate, with 2,6-di-*tert*-butyl-4-methylpyridine as an acid scavenger and a participating solvent, acetonitrile. Participating solvents have been used previously to increase the yield of β-glycosides in the presence of a non-participating group at the 2-position. Unfortunately this approach has not been profitable for the synthesis of β-mannosides likely due to the steric hindrance involved in making a 1,2-cis linkage.[146] The use of ulosyl bromide donors and a participating solvent proved effective for the synthesis of the β-mannosides explored here. The reaction gave the desired trisaccharide in 40-45% yield and 10% yield of the α-gluco epimer together with a significant portion of the 3,4-di-*O*-benzyl-1,6-anhydro-β-D-mannopyranose.[55] The α-gluco epimer likely arose from formation of the α-uloside which is reduced to the gluco

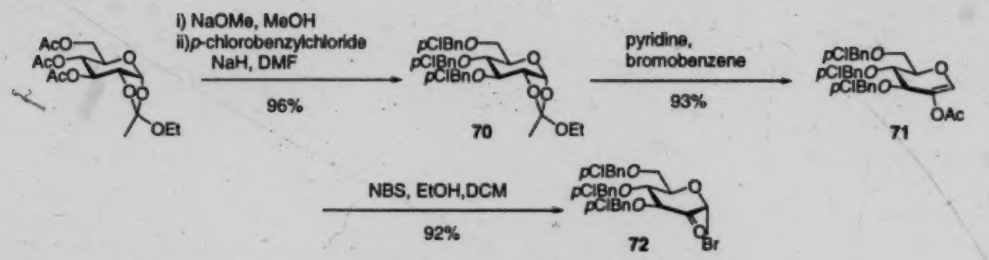
product. It was hypothesized that the 3,4-di-*O*-benzyl-1,6-anhydro-β-D-mannopyranose must form via attack at the anomeric center by O-6, followed by loss of the benzyl group (Scheme 3.3).

p-Chlorobenzyl ethers are more stable to acidic hydrolysis than their parent structures and thus stabilization of the protecting groups by installing *p*-chlorobenzyl ethers would likely disfavor the formation of this anhydro sugar side product and in turn increase the yield of the reaction.[147] The *p*-chlorobenzyl protected donor was synthesized analogously to the previously prepared benzyl protected ulosyl bromide (Scheme 3.4).[56]



Scheme 3.3. Synthesis of (1-2)-linked β-mannosyl trisaccharide using benzyl protecting groups

Standard deacylation of the readily available 3,4,6-tri-*O*-acetyl-(exo-ethoxyethylidene)-α-D-glucopyranose,[143] followed by Williamson ether synthesis with *p*-chlorobenzyl chloride gave the *p*-chlorobenzyl protected orthoester (**70**). Subsequent thermal rearrangement in bromobenzene gave a high yield of the acetoxyglycal (**71**) attesting to the increased stability of the *p*-chlorobenzyl ethers. Subsequent treatment with *N*-bromosuccinimide and ethanol in dichloromethane gave the desired ulosyl bromide (**72**) in adequate purity for glycosylations (Scheme 3.4).



Scheme 3.4. Preparation of *p*-chlorobenzyl protected ulosyl bromide

Glycosylation with this donor (**72**), in the presence of silver triflate and 2,6-di-*tert*-butyl-4-methylpyridine in acetonitrile, followed by reduction with L-Selectride gave a 60-65% yield of the desired trisaccharide (**73**) along with 15% of the corresponding α-gluco epimer (**74**), which were easily separated by column chromatography (Scheme 3.5). The *p*-chlorobenzyl protecting groups also imparted superior chromatographic qualities to the trisaccharide (**73**) than were observed with the benzyl protected trisaccharide thereby facilitating its purification.

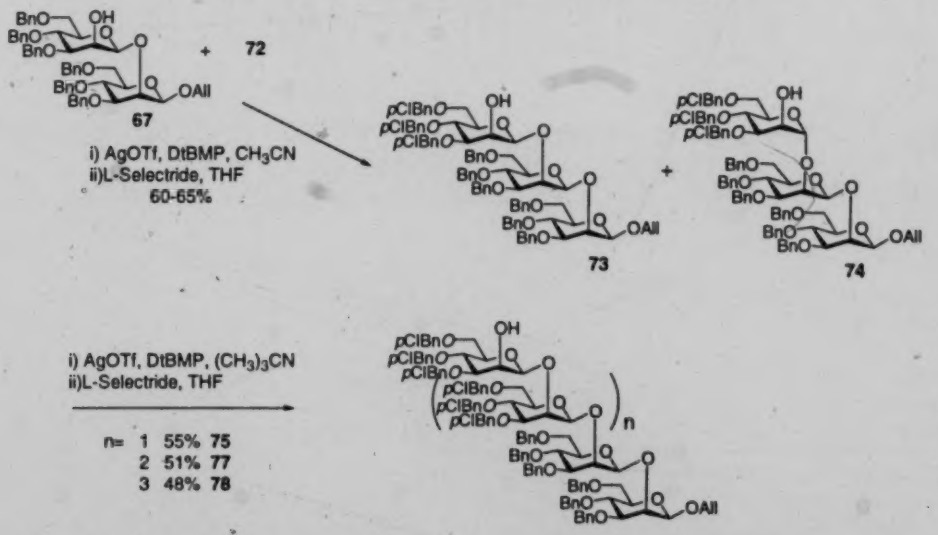


Figure 3.5. Synthesis of 1,2-linked β-mannopyranosyl hexasaccharide using *p*-cholorobenzyl protecting groups

Attempted introduction of the fourth β-mannopyranosyl residue was met by the observation of an interesting and unexpected product (Scheme 3.6).

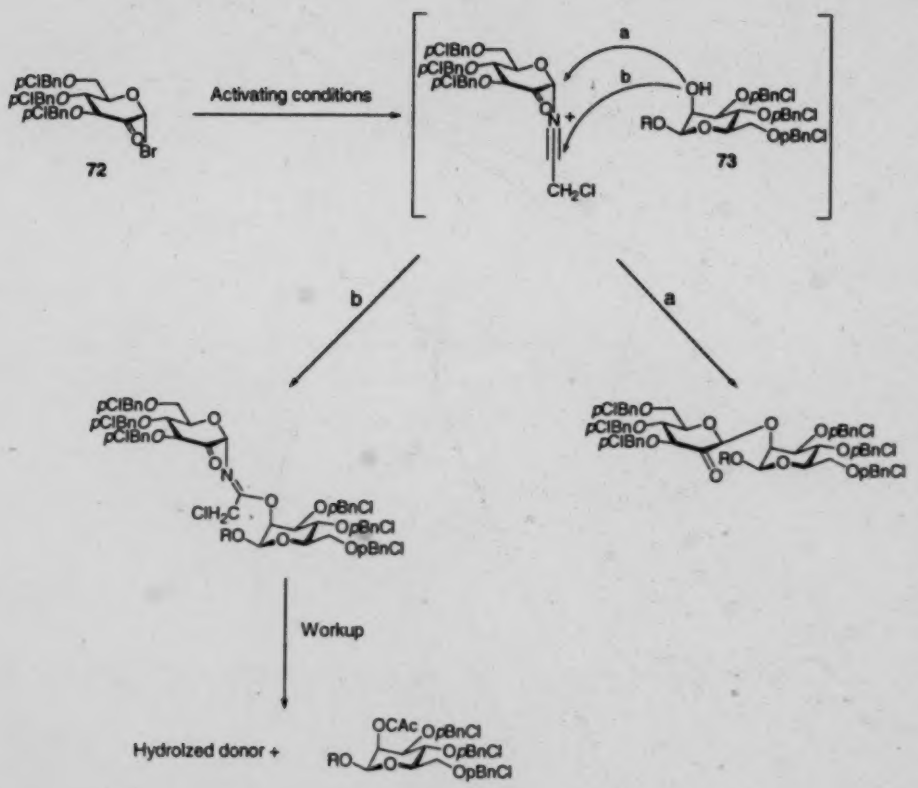


Figure 3.6. Hypothesis for the formation of the acylated glycosdic intermediate

Using the same conditions as those employed to make the trisaccharide, up to 20% of the unexpected 2-*O*-acetyl trisaccharide acceptor was isolated. We hypothesized that this product must result from attack of the acceptor on the nitrile carbon of the proposed α-nitrilium intermediate (path b), instead of reaction at the anomeric center of the donor (path a). This postulate was supported by the isolation of the chloroacetylated acceptor when chloroactonitrile was employed as the solvent (Figure 3.6). Although reaction at the nitrile carbon of nitrilium intermediates has been seen with water and carboxylic acids[146] only one example of an acylated acceptor has been isolated as a result of using nitriles as a participating solvent.[148] In this case a highly reactive glycosylidene carbene was used as the glycosyl donor and a weakly reactive

fluorinated alcohol as the acceptor. In this example the resulting side product must be favored due to a sterically hindered acceptor as well as the electron deficient nitrilium uloside.

When the sterically more hindered pivaloyl nitrile was chosen as the solvent, disfavoring attack at the nitrile carbon, the glycosidic linkage was synthesized in 55% yield to give the desired tetrasaccharide **75**, and 12% of the α-gluco epimer. This tetrasaccharide could then be elaborated via two sequential glycosylations to the hexasaccharide. Each glycosylation gave a similar yield and selectivity suggesting the environment of the acceptor hydroxyl is similar in the tri, tetra and pentasaccharides.

The allyl groups of the compounds were then modified before deprotection. It was initially thought dissolving metal conditions would remove the benzyl ether protecting groups and leave the allyl glycoside intact. Unfortunately dissolving metal conditions also lead to removal of the allyl protecting group and a complex mixture resulted. Thus a terminal amine was installed before deprotection via photoaddition of 2-aminoethanethiol to the allyl glycoside, in acceptable yields, ranging from 73-78%. Previously this reaction has only been accomplished with acyl protected oligosaccharides or on deprotected substrates. In the presence of the benzyl protecting groups it was necessary to use long wave ultra violet irradiation, 365 nm.[142] This slowed the reaction significantly but shorter wavelength irradiation, 254 nm, resulted in a complex mixture of products. These amines could then be deprotected under dissolving metal conditions to give the desired glycosides in good yield (Scheme 3.7).

Removal of the *p*-chlorobenzyl protecting groups and reduction of the allyl group to give the propyl glycosides was troublesome. Using standard hydrogenation conditions with palladium on carbon, only low yields of the desired product were obtained. It has been reported that using elevated pressures this reaction can be achieved[147] but due to the limited amount of material it was decided to reduce the allyl group with diimide and proceed with the same dissolving metal conditions which worked well for the amine terminated oligomers. (Scheme 3.7)

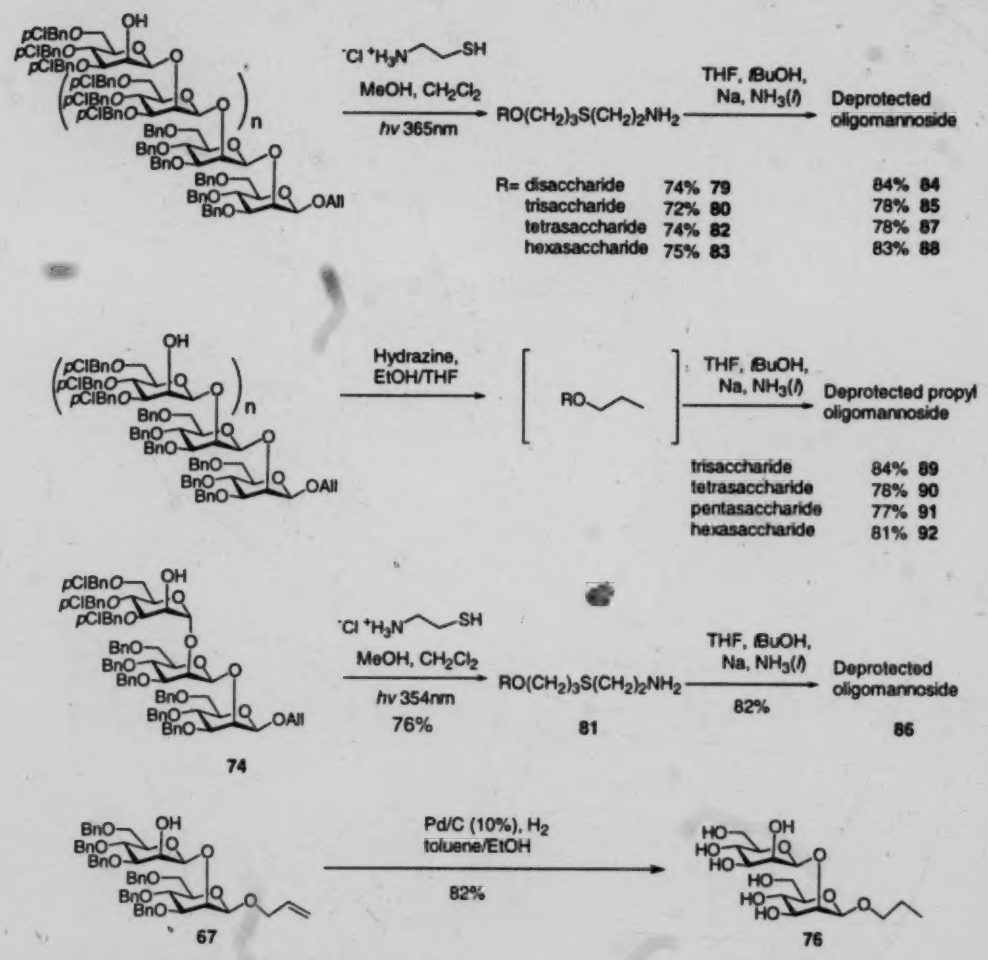


Figure 3.7. Deprotection of (1-2)-β-mannopyranans

Further studies on the interesting conformations of these compounds and their application as glycoconjugates are reported in Chapters 5 and 6.

3.3 Further work

To put this new approach on the use of ulosides into context, donor **72** should be used with an acceptor, which is often targeted for the synthesis of the *N*-linked core oligosaccharide, methyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside. Further optimizations

towards improving the anomeric stereoselectivity of the reaction should also be addressed. Changing the order of addition may allow formation of the α-nitrilium ion at low temperature prior to the addition of the alcohol, leading to increased selectivity. This would be analogous to approaches used with sulfoxide[57] and imidate[149] donors that have been used to form active glycosyl triflates at low temperature to increase the selectivity of β-mannoside synthesis.

Chapter 4

Synthesis of a thio-linked mimetic of the (1→2)-β-D-mannotetrose of the *Candida albicans* antigen

4.1 Introduction

4.1a Background

With the discovery of the increasing importance of carbohydrates in biological systems it is desirable to synthesize sugar mimetics for a variety of biological studies.[150] Replacing the glycosidic oxygen with a sulfur atom provides a metabolically stable oligosaccharide mimetic. This class of compounds was initially developed as glycosidase inhibitors having similar conformational properties to the native oligosaccharides while being resistant to cleavage.[151] These compounds have been co-crystallized with glycosidases to give insight into the active site of such enzymes as the *Trichodera reesei* cellulase.[152] With the advent of new applications of carbohydrates as inhibitors of binding events and metabolic probes, thioglycosides have arisen as the mimetics of choice for biologically important oligosaccharides. Thio-linked analogs of many interesting oligosaccharides have been synthesized and their biological and conformational properties are being investigated.[153,154]

4.1b Synthesis of thioglycosides

A variety of methods are available to synthesize thioglycosides, which capitalize on the inherent nucleophilicity of thiols when compared with alcohols. It is possible to generate 1-thiolate glycosyl donors and accomplish an S_N2 displacement of a leaving group on a glycosyl acceptor.[155] This approach has been used to synthesize the usually challenging β-mannoside linkages[156] and has recently been adapted for use on the solid phase.[157] These methods generally employ the powerful triflate leaving group to facilitate the reaction.

The classical approach of attack at the anomeric center can be achieved by an S_N2 displacement of a glycosyl halide, which has been productive for forming α sialylosides[158] or via Lewis acid activation of a glycosyl imidate followed by thiol attack.[159] (Figure 4.1)

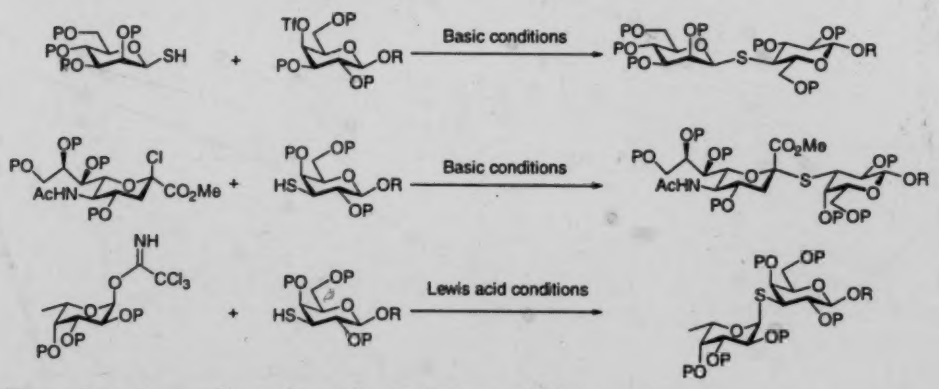
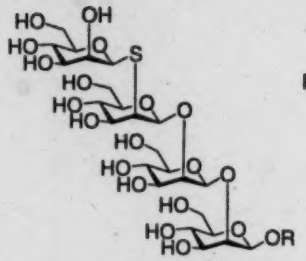


Figure 4.1. Approaches to the synthesis of thioglycosides

4.1c Scope of project

A (1→2)-β-mannopyranotetrose was synthesized containing a terminal thio-glycosidic linkage (Figure 4.2). This compound will allow determination of the possibility of using thio-linked glycans as mimics of the parent structures when they are presented to the immune system. Conjugates of this compound should be more metabolically stable to *exo*-β-mannosidases and therefore better immunostimulants.



R= functionality to facilitate glycoconjugate formation

Figure 4.2. Synthetic mimetic of a structures found in the phosphomannan of *C. albicans*

The conformation of this thioglycoside analogue was to be compared to the O-linked tetrasaccharide to gain insight into how well this thioglycoside mimics the natural structure.

4.2 Synthesis of the thioglycoside analogue

4.2a Retrosynthetic analysis

As was successful for the synthesis of the parent analogue **75,** the reducing terminal allyl glycoside would be used to facilitate conversion to either the propyl glycoside via reduction or the amine terminated thioglycoside through photoaddition.

Early studies on the feasibility of forming the β-thiomannoside indicated direct displacement with an anomeric thiolate was not feasible on the allyl 3,4,6-*O*-benzyl-2-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside, available directly from **65**, since only the α-thioglycoside could be obtained. Thus it was thought necessary to install the thiol on the trisaccharide and displace an anomeric leaving group. The previously synthesized ulosyl bromide **72** was chosen as the glycosyl donor in this reaction. The displacement of a 2-chloro sialic acid derivatives, which has shown success is analogous to displacement of the bromide of a uloside, in that both halide leaving groups are α to a carbonyl.[159] Furthermore ulosyl bromides had previously been shown to react with simple thiols in high yield.[55]

The thiol was introduced to the trisaccharide using a nucleophilic inversion of the triflate. In order to avoid the possibility of ring contraction, a 4,6-*O*-benzylidene was installed at the

terminal β-D-glucopyranoside residue. An orthogonal protecting group would also be required at the C-2 terminus to facilitate formation of the required triflate for nucleophilic inversion. Fortunately this coincides well with the requirement of a participating group for the synthesis of the terminal β-glucopyranoside. The synthesis of this trisaccharide would take advantage of the previously synthesised disaccharide **67** and require the imidate **96** as a glycosyl donor. This glycosyl donor could be synthesised in 4 steps from the known 3-*O*-benzyl-D-glucopyranose. This compound was available on a large scale from commercial diacetone glucose via a one-pot synthesis (Figure 4.3).[160]

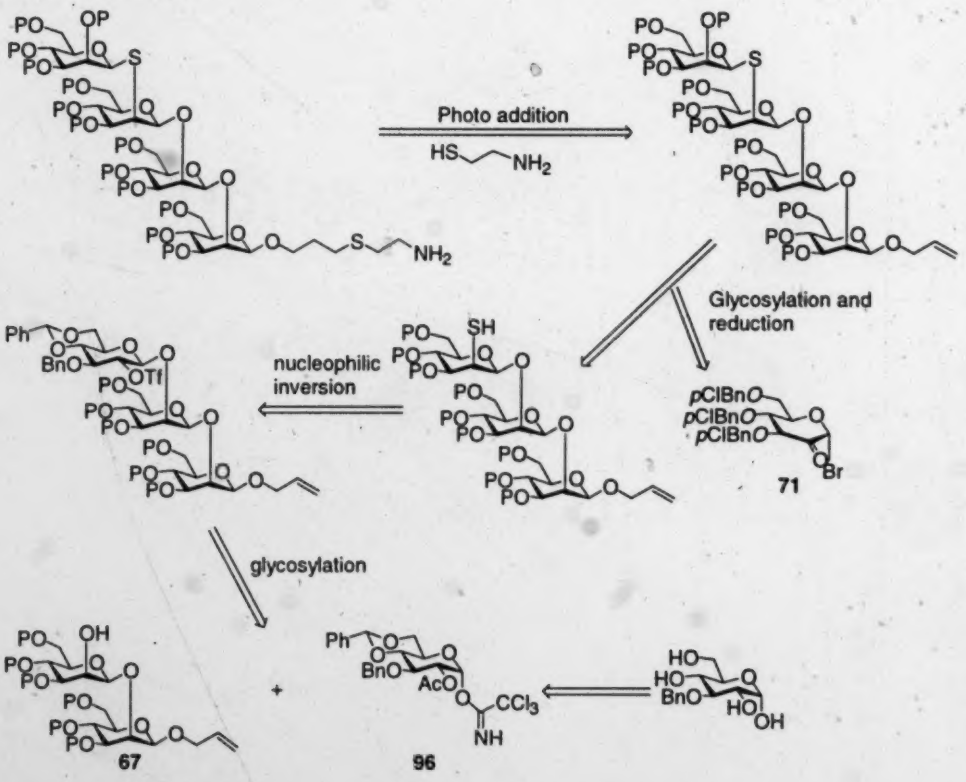
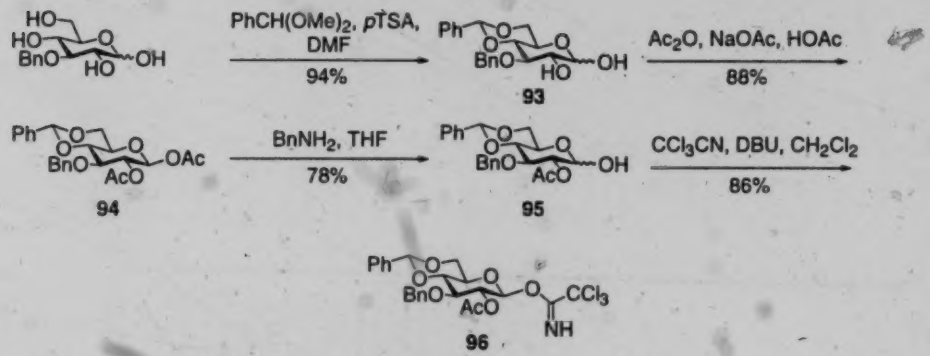


Figure 4.3. Retrosynthetic analysis of (1-thio-mannopyranosyl)-(1-2)-β-D-mannopyranotriose

4.2b Synthesis of the glucosyl donor

3-*O*-benzyl glucose was treated with benzaldehyde dimethyl acetal in dimethyl formamide with a catalytic amount of acid to give the benzylidene protected derivative **93**. This solution was slowly concentrated under vacuum to remove methanol. More vigorous conditions, including the use of acetonitrile as a solvent, resulted in considerable formation of other possible benzylidene rings. The synthesis of compound **93** was recently reported using benzaldehyde and zinc chloride in 54% yield.[161] The diol was acetylated to give **94**, and the anomeric acetate could be selectively removed with benzylamine in THF giving the hemiacetal **95**.[162] Generation of the trichloracetimidate under standard conditions gave the glycosyl donor **96** (Scheme 4.1).[163]

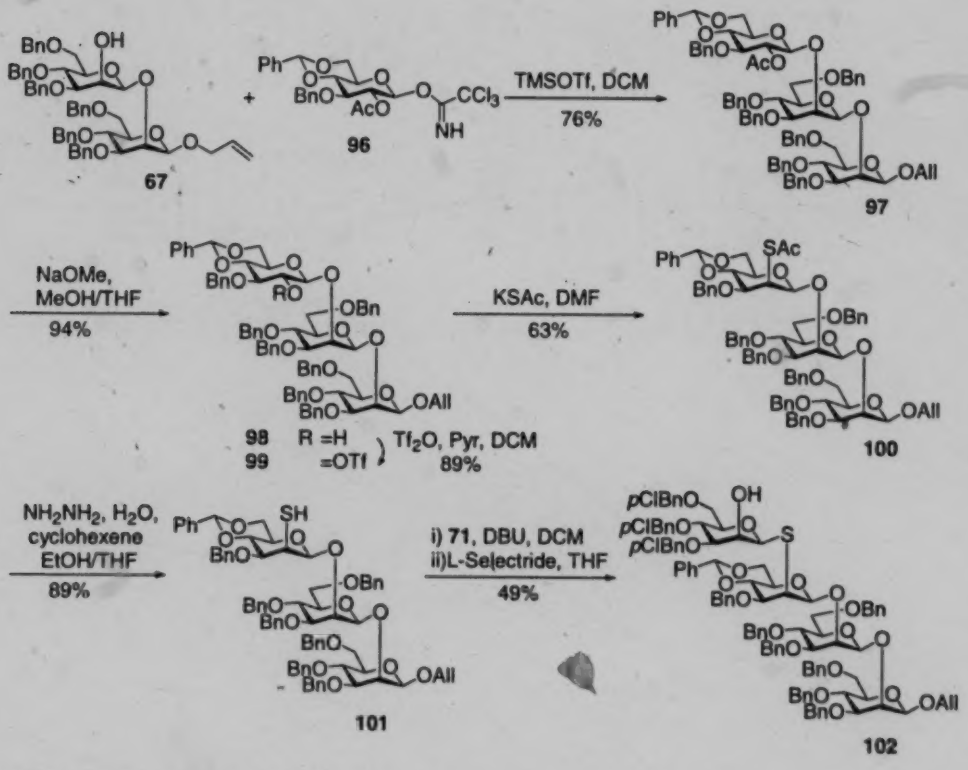


Scheme 4.1. Synthesis of glucopyranosyl imidate donor

4.2c Synthesis of thio-linked tetrasaccharide

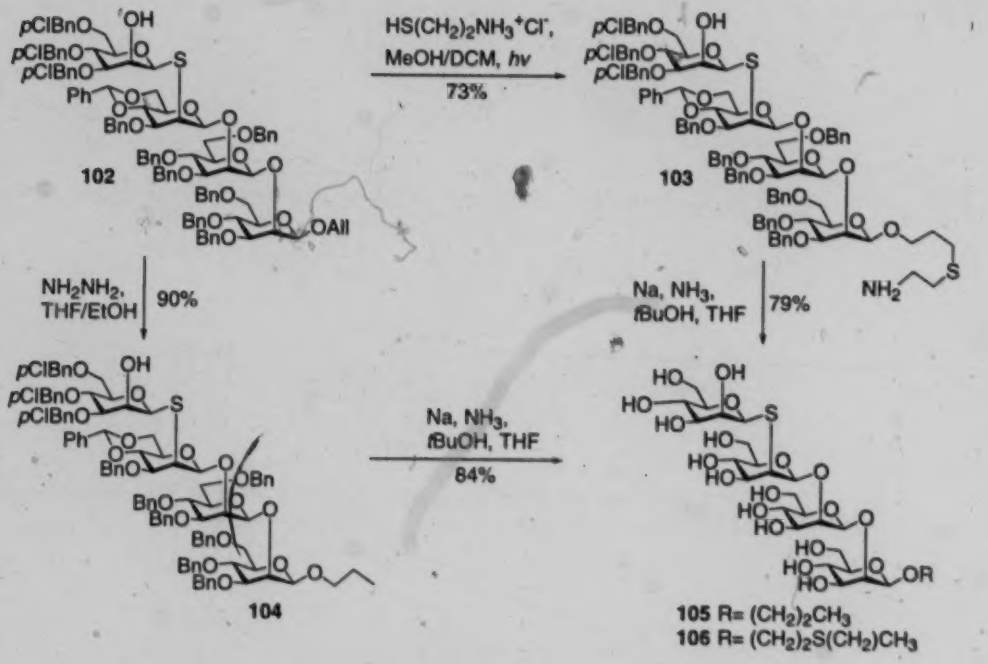
Formation of the trisaccharide (**97**) was accomplished in good yield from the imidate donor **96** and the disaccharide acceptor **67** using a minimal amount of TMSOTf in dichloromethane at room temperature. The 2-*O*-acetate of the trisaccharide could then be removed using sodium methoxide to give the alcohol **98** in a solution of methanol and THF, to ensure the solubility of the hydrophobic starting material. Introduction of the triflate under standard conditions gave the leaving group functionalized trisaccharide **99** necessary for introduction of a thioacetate (**100**). This reaction was somewhat troublesome, requiring elevated

temperatures and the yields could not be improved by the introduction of crown ether. Removal of the acetate proceeded smoothly to give the thiol **101**, which was surprisingly stable to atmospheric oxidation requiring no special handling. The thiol was condensed with the ulosyl bromide to give after reduction the desired tetrasaccharide, **102**, as well as the α-gluco epimer after reduction. This suggests that some β-ulosyl bromide may have been present in the reaction. It was not visible by NMR spectroscopy of the starting ulosyl bromide but may be formed via halide exchange analogous to the mechanism for formation of α-glycosides (Section 1.2c). Perhaps use of the ulosyl chloride, which should undergo halide exchange more slowly, decreased reaction temperatures, or the use of a different solvent would increase the selectivity of this reaction. Unfortunately time did not allow optimisation of these conditions.



Scheme 4.2. Synthesis of (1-thio-β-D-mannopyranosyl)- (1-2)-β-D-mannopyranotriose

The tetrasaccharide was elaborated under the same conditions as the previously formed 3-(2-aminoethylthio)-propyl glycosides. The thioglycoside did not appear to complicate the photoaddition of the thiol giving **103** in reasonable yield. The allyl glycoside was reduced with a solution of hydrazine hydrate, which forms diimide under atmospheric oxidation, selectively reducing the double bond giving **104**. Dissolving metal conditions efficiently removed the benzyl and *p*-chlorobenzyl protecting groups to give the desired tetrasaccharides, **105** and **106**, (Scheme 4.3).



Scheme 4.3. Deprotection of (1-thio-β-D-mannopyranosyl)-(1-2)-β-D-mannopyranotriose

4.3 Conclusion

This route, although not optimised, provides a viable path to the target compounds **105** and **106** which will be used for studying the response of the immune system to thioglycoside mimetics as well as its conformation.

Chapter 5

Conformational analysis of (1→2)-β-mannopyranosyl tri, tetra and pentosides and comparison to (1-thio-β-D-mannopyranosyl)-(1→2)-β-D-mannopyranotriose using NMR and molecular modelling

5.1 Introduction

5.1a Background

In order to understand and explain the varied functions and specificities of complex oligosaccharides it is necessary to have insight into their three dimensional structures, since this will govern the recognition by protein receptors and enzymes. [164] NMR and molecular modelling have provided useful tools to this end.[165] It is now generally accepted that oligosaccharides do not exist as rigid structures but are best represented by an ensemble of low energy conformations.[166] Molecular simulations provide a model of an oligosacharide's behaviour for comparison with conformationally averaged NMR data, and taken together they provide insight into the range of explored conformations by an oligosaccharide.

Conformational studies usually begin with the complete assignment of the ^{1}H NMR spectrum, through the use of two dimensional homonuclear and heteronuclear experiments. Unlike proteins, isotopically enriched carbohydrates are seldom available, limiting the number of heteronuclear experiments that can be easily applied. The use of gradient COSY, and TOCSY

experiments are usually sufficient to assign the majority of the ^1H signals, although heavy overlap in the 3.0-4.0 ppm region of the spectrum is often a severe problem. To overcome this obstacle a variety of two and three dimensional NMR techniques have been developed.[178] After a complete assignment of ^1H resonances, dipolar and scalar couplings can be measured and used as restraints to build a model of the oligosaccharide.

Scalar coupling constants can be used in conjunction with Karplus relationships to determine the relative positions of coupled nuclei around the pyranose ring and the average conformation of the oligosaccharide through coupling across the glycosidic linkage. For most cases, homonuclear proton $^3J_{1,2}$ coupling constant reveals the stereochemistry about the anomeric center, provided the stereochemistry at C-2 is known. Thus with glucopyranosides the β-anomer will have coupling constants in the 6-8 Hz range and the α-anomer in the 2-4 Hz range.[167] With mannopyranosides, and like stereochemistries, this interpretation is difficult due to small $^3J_{1,2}$ coupling constants that are observed for both the α and β anomers. This ambiguity can be resolved by measuring one bond heteronuclear the $^1J_{C1,H1}$ coupling constant. For α anomers this is larger than 165 Hz and less than 165 Hz for β anomers.[114] Correlations of the long range scalar coupling constants $^3J_{COCH}$ and $^3J_{COCC}$ across the glycosidic linkage with a Karplus type relationship have also been developed, allowing measurement of the average angle across the glycosidic linkage.[168,169] Unfortunately, these measurements take extended periods of NMR instrument time, even with high sample concentrations, and are best suited to isotopically enriched samples.

The recognition element (epitope) of an oligosaccharide is usually comprised of 3-6 hexose residues. Consequently, many interesting carbohydrates have correlation times that result in little or no nuclear Overhauser effect (NOE) signal at ambient temperature. The use of rotating-frame Overhauser effect (ROE) techniques, that give similar information but do not suffer from null signals at intermediate correlation times, allow the quantification of H-H distances up to

approximately 4 Å. Unlike proteins, oligosaccharides rarely contain enough NOE contacts to define their conformations unambiguously. New techniques, which slow the exchange of hydroxyl protons through the use of organic solvents and low temperatures, are providing a greater number of contacts through observation of hydroxylic proton resonances.[170]

It is important to realize that ROE distances are averaged over the conformations adopted by the oligosaccharide, since the time scale for the ROE measurement is of the order of a millisecond, while the time scale of conformational exchange is on the order of molecular tumbling (nanosecond).[171] Thus it is common to observe ROE contacts in a molecule that arise from two distinct conformations and cannot be simultaneously satisfied with any one rigid model. Moreover, if strict distance restraints are used from this conformationally averaged ROE data, erroneous or virtual conformations will result. Dynamic models that can account for conformational averaging give a more accurate representation of the oligosaccharide's behaviour.[172]

Molecular modelling provides a method to interpret the gross properties of an oligosaccharide in terms of the relative potential energies of each conformation it explores. The calculation of these potential energies requires a forcefield that contains parameters for all the properties of the molecular system such as torsional strain, or electrostatics of certain bonding combinations. There are now many force fields that have been parameterized for carbohydrate modelling, generally by including terms for the anomeric and exo-anomeric effect, including: AMBER,[173] CHARMm,[174] GROMOS,[175] MM3[176] and Tripos.[177] No general consensus can be found in the literature as to which forcefield yields the best models of oligosaccharide behaviour.[178] However, a single systematic study on methyl α-lactoside has shown that MM3 gave the best fit with experimental data when a series of forcefields were compared.[179]

Two methods have generally been used to explore the conformational energy surfaces of carbohydrates. The torsional angles can be varied in a systematic way and the energy for each conformation calculated giving a potential energy surface for each set of dihedral angles. This

type of calculation is very computationally intensive for even reasonably sized oligosaccharides. For example, a tetrasaccharide has 8 glycosidic torsional angles, if each is varied in 10° increments 36^8 full minimization calculations are required to define the energy surface. But, once this has been accomplished it provides the best basis to calculate the gross physical properties of a system using a Boltzmann distribution. Recently, other algorithms for exploring the energy surfaces have been developed that are less systematic but still define the surface.[180] Hopefully these algorithms will be made available in the near future so groups less specialised in theoretical approaches may apply them.

The use of molecular dynamics calculations is a different approach to producing a tangible model of an oligosaccharide.[181] Using this approach the conformations explored by an oligosaccharide over a short time period are observed by applying Newton's laws of motion to the atoms of the molecular system. The gross physical properties of the system are then calculated from the time averaged population of conformers. Good correlations between experimental and theoretical data have been obtained for the gross physical properties of many oligosaccharides using this method. This approach is somewhat biased by the starting structure, as it is rarely computationally possible to follow the system for a long enough period of time for the model to obtain an equilibrium between conformations that are separated by high energy barriers. Thus it is important to begin the calculation with the global minimum energy conformation to obtain realistic results.

The Amber forcefield is integrated into a user friendly modelling package, Insight II, and is available from Biosym Technologies, making molecular dynamics analysis readily available. Ideally it would be possible to include explicit solvent molecules in these calculations, but these many-bodied problems cannot easily be solved with readily available computational resources.

The definitions of the torsional angles around the glycosidic linkage, which will be discussed in this chapter, are shown in Figure 5.1. The dihedral angles are ϕ^H (H1B-C1B-O1B-

C2A) and ψ^H (C1B-O1B-C2A-H2A) and the residues are labeled alphabetically from the reducing terminus. The abreviations ϕ and ψ will be used in the place of ϕ^H and ψ^H.

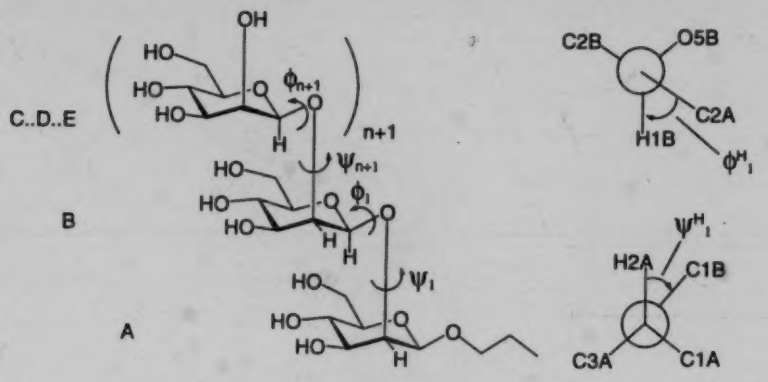


Figure 5.1. Definitions of glycosidic torsional angles.

Due to the exo-anomeric effect it is common for glycosides to adopt a ϕ angle near 60° or –60° degrees, depending on the stereochemistry at the anomeric center. This geometry places the lone pairs on the exocyclic oxygen anti to the C-O bond in the pyranose ring, which is required for stabilization by the exo-anomeric effect. The ψ angle is usually found in the region of 0° but can in principle adopt a value of 0°-180°. At an angle of 0° the protons are "*syn*" across the glycosidic linkage and this is known as the *syn*-conformation. At an angle of 180° this is known as the *anti*-conformation and is usually higher in energy due to steric interactions.

5.1b Scope of investigation

The NMR spectral data for the oligosaccharides **89**, **90**, **91**, **92** and **105** will be discussed, and the application of molecular dynamics will be used to generate a model for the conformations of these unique homopolymers.

5.2 NMR of (1→2)-β-mannopyranan tri, tetra and pentose

5.2a Assignment of ^{1}H and ^{13}C spectra for (1→2)-β-mannopyranan tri, tetra and pentose oligomers

Considering the homo-polymeric nature of these oligosaccharides it was surprising to find excellent signal dispersion in the ^{1}H NMR spectra. Other carbohydrate based homopolymers such as kojitetraose ((1→2)-α-glucopyranotetrose),[182] maltoheptose ((1→4)-α-glucopyranotetrose),[183] and (1→2)-α-mannopyranotetrose show extensive overlap in their ^{1}H NMR spectra. Of particular note, the Brucella A polysaccharide antigen, (1→2)-α-4,6-dideoxy-4-formamido-mannopyranose),[184] and the Brucella polysaccharide B, (cyclic (1→2)-β-glucopyranose 17-24 residues), which show nearly coincident ^{1}H and ^{13}C signals for all the pyranose rings. This suggests that (1→2)-β-mannopyranan oligomers are more ordered and conformationally distinct than other homo-oligomers, even for short sequences, and this perhaps influences their biological properties.

The ^{1}H NMR spectra were assigned using a combination of gradient enhanced COSY and TOCSY experiments. These experiments have been shown to be excellent in detection of correlations between protons with small $^{3}J_{HH}$ coupling constants.[185] The exceptional signal dispersion for the tetrasaccharide and the ease with which it could be assigned can be seen in the TOCSY spectra in Figure 5.2.

Isolated (1→2)-β-D-mannopyranan oligomers have previously been investigated independently by the groups of Suzuki[186] and Strecker.[187] They have assigned the ^{1}H and ^{13}C spectra of oligomers as long as seven residues. Direct comparisons of the data for the H-1, H-2, H-3, and H-4 resonances are found in Table 5.1. These signals were chosen because they have excellent dispersion within all the oligomers analyzed. Heavy overlap for H-5 and H-6's was observed for all the compounds.

There are discrepancies in the literature data that cannot be easily attributed to any systematic error. It is possible that the isolation procedures differed and the presence of contaminants is affecting the NMR data. It is well known that the presence of metal ions, which can be chelated by hydroxyl groups, affect the chemical shift of ring protons and carbon nuclei.[188] Comparison between the oligosaccharides synthesized here and those isolated must be done cautiously, as it is expected that the reducing terminal sugar assignments would be significantly different, as the naturally isolated material is a free hemi-acetal, and the data presented for all the synthetic compounds are for propyl glycosides.

Table 5.1. Comparison of literature ^{1}H NMR data and that of **89**, **90**, **91** and **92**

[d]	H-1			H-2			H-3			H-4		
	Ref. 186[a]	Ref. 187[b]	This work[c]	Ref. 186[a]	Ref. 187[b]	This Work[c]	Ref. 186[a]	Ref. 187[b]	This Work[c]	Ref. 186[a]	Ref. 187[b]	This Work[c]
M_3												
A	5.27	4.98	4.72	4.10	4.16	4.23	3.90	3.67	3.67	3.61	3.50	3.48
B	4.84	4.91	4.91	4.27	4.41	4.37	3.66	3.67	3.64	3.61	3.61	3.59
C	4.85	4.95	4.95	4.15	4.15	4.15	3.62	3.26	3.61	3.57	3.56	3.57
M_4												
A	5.27	4.99	4.73	4.11	4.18	4.24	3.90	3.68	3.67	3.59	3.48	3.48
B	4.83	4.88	4.89	4.24	4.39	4.40	3.69	3.70	3.68	3.51	3.52	3.50
C	4.92	5.04	5.04	4.40	4.38	4.35	3.63	3.64	3.64	3.59	3.60	3.60
D	4.91	4.93	4.94	4.15	4.15	4.16	3.61	3.62	3.62	3.57	3.57	3.57
M_5												
A	5.27	4.99	4.72	4.11	4.18	4.23	3.91	3.69	3.68	3.60	3.47	3.48
B	4.83	4.89	4.89	4.25	4.39	4.34	3.70	3.70	3.67	3.50	3.51	3.49
C	4.91	5.03	5.00	4.39	4.39	4.38	3.66	3.64	3.66	3.50	3.59	3.58
D	5.01	4.92	5.03	4.37	4.41	4.38	3.63	3.69	3.63	3.59	3.50	3.51
E	4.94	4.95	4.95	4.15	4.16	4.15	3.63	3.27	3.62	3.58	3.57	3.56
M_6												
A	5.27	4.99	4.77	4.11	4.18	4.24	3.91	3.68	3.68	3.60	3.57	3.48
B	4.83	4.89	4.90	4.25	4.39	4.34	3.70	3.71	3.68	3.51	3.50	3.50
C	4.92	5.01	5.02	4.40	4.37	4.40	3.67	3.69	3.67	3.49	3.59	3.51
D	4.99	5.01	5.02	4.36	4.39	4.37	3.66	3.66	3.67	3.50	3.51	3.50
E	5.03	4.94	5.06	4.37	4.41	4.40	3.64	3.67	3.65	3.59	Nd	3.59
F	4.94	4.96	4.96	4.15	4.16	4.16	3.62	3.63	3.63	3.58	3.48	3.57

[a] Reference Suzuki *et al.*[186]
[b] Reference Strecker *et al.*[187]
[c] Data collected in this work
[d] residues labeled alphabetically from reducing terminus: M_3 (1→2)-β-D-mannopyranotriose; M_4 (1→2)-β-D-mannopyranotetraose; M_5 (1→2)-β-D-mannopyranopentose; M_6 (1→2)-β-D-mannopyranohexose

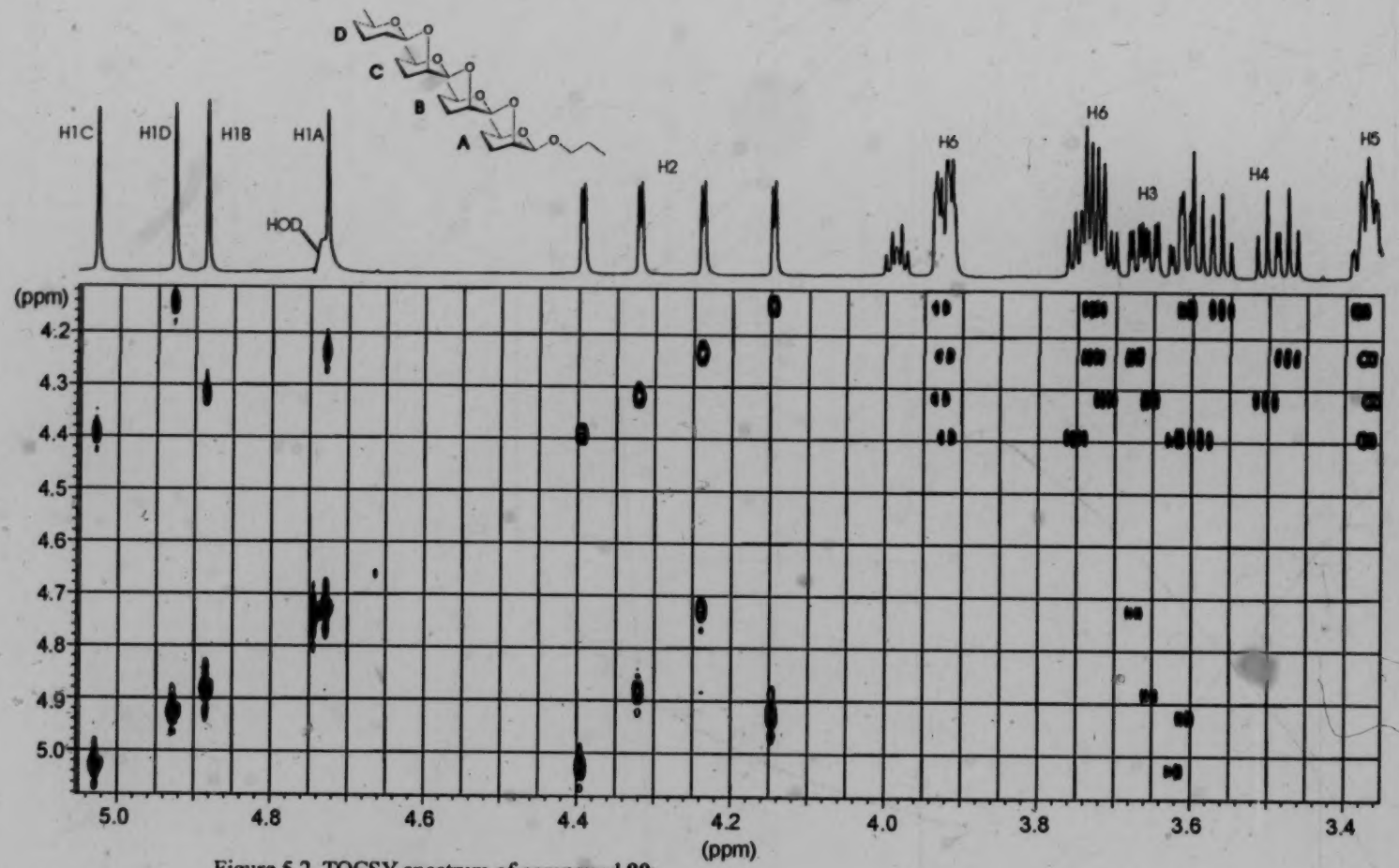


Figure 5.2. TOCSY spectrum of compound **90**.

The ^{1}H assignments of the internal residues agree more closely with those collected by Strecker *et al.*[187] than those of Suzuki *et al.*[186] The greatest differences are found in the anomeric signals of the penta and hexasaccharides. The two literature references have the assignments of H-1C and H-1D reversed and they are strikingly different to those values found in this study. It is possible that the hemi-acetal of the reducing terminus is affecting these signals leading to the observed 0.1 ppm difference. This is likely given the three dimensional structure of this oligomer which brings these residues close in space.

Table 5.2. Comparison of literature ^{13}C data and of **89**, **90**, **91** and **92**

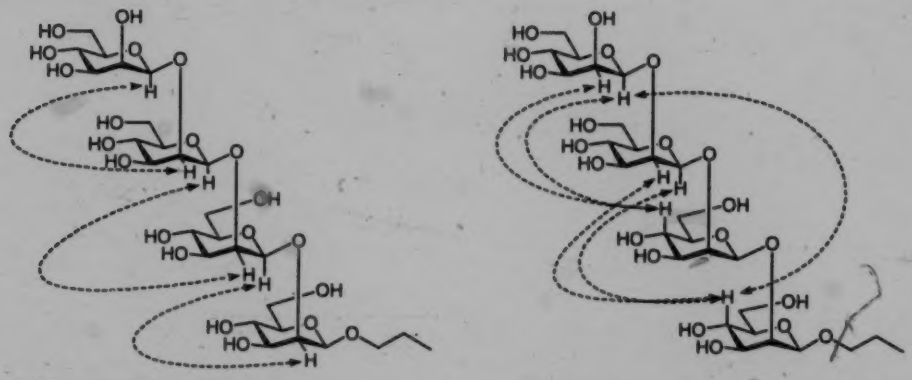
[d]	**H-1**			**H-2**			**H-3**		
	Ref. 186[a]	Ref. 187[b]	This Work[c]	Ref. 186[a]	Ref. 187[b]	This Work[c]	Ref. 186[a]	Ref. 187[b]	This Work[c]
M_3									
A	92.9	94.9	100.9	79.5	80.9	79.7	70.2	73.5	73.8
B	99.9	102.3	101.9	79.5	79.4	79.0	73.1	73.4	73.1
C	101.8	102.1	101.6	71.3	71.8	71.3	73.9	74.3	73.0
M_4									
A	93.0	94.8	101.8	79.8	81.3	80.1	70.1	73.3	72.8
B	100.2	102.5	102.0	80.3	80.3	79.9	72.8	73.2	72.8
C	102.1	102.4	102.0	79.3	79.6	79.2	73.2	73.6	73.2
D	101.9	102.3	101.8	71.3	71.7	71.3	73.9	74.3	73.8
M_5									
A	93.0	94.8	100.8	79.8	81.4	80.1	70.1	Nd	Nd
B	100.2	100.5	102.1	80.6	80.9	80.1	72.7	73.1	Nd
C	102.3	102.4	102.2	79.9	80.3	79.8	72.9	73.6	Nd
D	102.1	102.7	101.9	79.4	79.8	79.3	73.2	73.3	Nd
E	101.9	102.3	101.8	71.3	71.7	71.3	73.9	74.2	73.8
M_6									
A	93.0	94.9	100.8	79.8	81.3	80.0	70.1	70.4	Nd
B	100.2	100.5	102.2	80.6	80.9	80.0	72.7	73.1	Nd
C	102.3	100.6	102.1	80.2	80.4	80.0	72.8	73.6	Nd
D	102.2	102.4	102.1	80.0	79.8	79.9	73.0	73.4	Nd
E	102.0	102.7	101.9	79.4	80.5	79.3	73.2	73.2	Nd
F	101.9	102.3	101.8	71.3	71.4	71.3	73.9	74.3	73.8

[a] Reference Suzuki *et al.*[186]
[b] Reference Strecker *et al.*[187]
[c] Data collected in this work
[d] residues labeled alphabetically from reducing terminus: M_3 (1→2)-β-D-mannopyranotriose; M_4 (1→2)-β-D-mannopyranotetraose; M_5 (1→2)-β-D-mannopyranopentose; M_6 (1→2)-β-D-mannopyranohexose

The ^{13}C spectrum of the oligosaccharides were assigned from HMQC experiments. The data agree within experimental error with the literature data for the C-1, C-2 and C-3 atoms (Table 5.2) given the differences at the reducing terminus. Assignments of the other ^{13}C resonances would require extended acquisition times at the concentrations available from the synthesis.

5.2b Analysis of ROE contacts for (1→2)-β-mannopyranan oligomers

The unexpected dispersion in the spectra of the (1→2)-β-mannopyranosyl oligosaccharides suggested a well ordered three dimensional structure for these polymers. To investigate this structure further T-ROESY NMR experiments were employed.[189] The T-ROESY data were quantified for the tri, tetra, and pentasaccharides. Distance references were taken from average intra-residue H1-H5 contacts. Inter-residue distances were calculated based on the r^{-6} relationship between distance and ROE intensity.[192] The hexasaccharide had significant overlap of the important resonances and the ROE contacts from this structure were not quantified. Spectra for the tetra and pentasaccharides were obtained on the 800 MHz spectrometer at the NANUC facility. A surprising number of ROE contacts between non-contiguous residues were well resolved for the mannopyrans investigated.



ROEs across glycosidic linkage ROEs between non-contiguous residues

Figure 5.3. Observed interglycosidic ROE contacts in compound **90**

An example of the contacts observed for the tetrasaccharide is given in Figure 5.3. All of the interglycosidic contacts between the contiguous redsidues are present and easily quantified due to the signal dispersion of the H-1 and H-2 signals. The presence of contacts between H-4A and H-1C, H-2C and H-4B, H-1D and H-2D, and separated by two pyranose rings, H4A and H-1D are clearly observed. A portion of the T-ROESY spectrum for propyl (1→2)-β-pentamannopyranoside (**91**) is shown in Figure 5.4 to illustrate the observed signals between non-contiguous residues. Taken together these distance constraints infer a compact repetitive structure in this homopolymer with similar distances across the glycosidic linkage for all the oligomers (Table 5.3). The distances suggest the structure is not a result of steric interactions between distant non-contiguous, residues but represents a population of low energy conformations with similar torsional angles about the glycosidic linkages. If steric interactions were limiting the conformations of the oligomers, the shorter oligomers, lacking steric interactions between non-contiguous residues, would be expected to have distances that differed from those of the higher molecular weight counterparts.

Table 5.3 ROE contacts for (1→2)-β-mannopyranosyl tri, tetra and pentasaccharides

ROE contacts observed	**Distances calculated (Å)**		
Contacts across glycosidic linkage	**89**	**90**	**91**
H2A-H1B	2.26	2.25	2.24
H2B-H1C	2.29	2.24	2.21
H2C-H1D	-	2.29	Nq
H2D-H1E	-	-	2.25
Contacts separated by one residue			
H4A-H1C	3.32	3.14	3.22
H4A-H2C	2.86	2.78	2.72
H4B-H1D		3.09	3.12
H4B-H2D		2.68	2.65
H4C-H1E			3.15
H4C-H2E			2.83
Contacts separated by two residues			
H4A-H1D		4.13	3.71
H4B-H1E			4.20

Oligomers are labeled alphabetically from the reducing terminus
Nq not quantified due to overlap

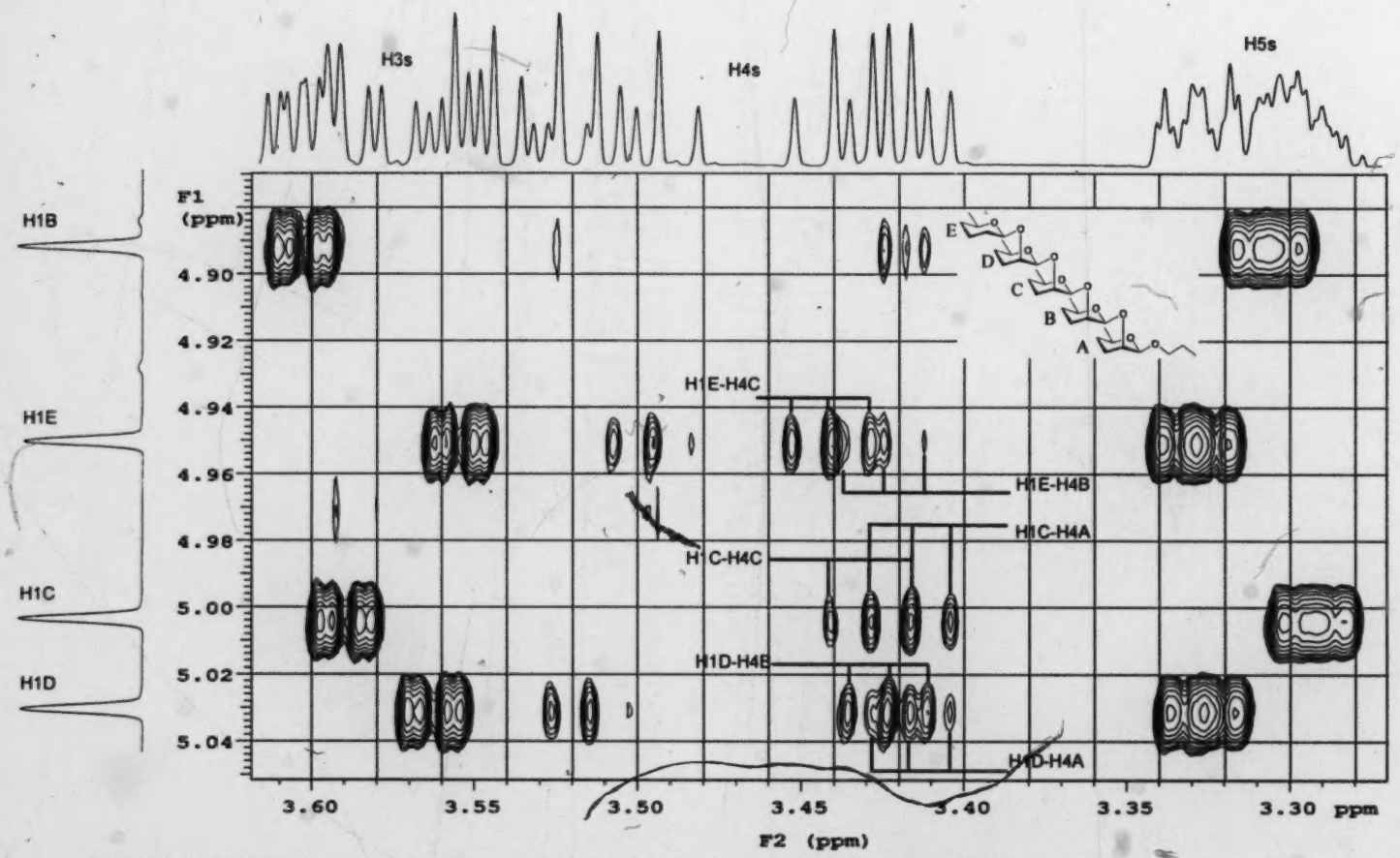


Figure 5.4. T-ROESY spectrum of pentasaccharide **91** showing long range ROE crosspeaks between non-contiguous residues

The presence of multiple ROE contacts between non-contiguous residues is very rare in oligosaccharides. Often it is possible to see a single interaction between non-contiguous residues in branched oligosacharides, but two well defined contacts is exceptional. The presence of ROE contacts between residues separated by two pyranose rings to our knowledge is unprecedented, and indicative of a very compact structure. Molecular dynamics simulations were undertaken to provide a model for this unique oligosaccharide.

5.3 Molecular dynamics simulations

5.3a Introduction

Molecular dynamics simulations of the structures were undertaken to provide a model to compare with the experimental ROE data. Using the Amber forcefield modified by Homans *et al.* for carbohydrates, and the Insight II modelling software, the methyl (1→2)-β-mannopyranosyl tri, tetra and pentasaccharides were modelled.[190] The methyl glycosides were chosen to simplify the calculations. These simulations began with a short, high temperature molecular dynamics run where the chair conformations of the mannopyranosyl rings were enforced by scaling the torsional energy term by 7. From this simulation ten structures were generated to provide starting points with random torsional angles about the glycosidic linkages and the exocyclic hydroxymethyl groups. These ten structures were each minimized to a low energy conformer by a simulated annealing protocol.[191] This involves running stepwise 1 ps molecular dynamics simulations starting at 500 K and cooling to 300 K in 50 K increments, followed by decent in 10 K steps to 10 K and finally down to 5 K. Each annealed structure was then minimized further using a steepest gradient method. The comparison of the ten structures generated by this protocol gave insight into the conformational minima of the molecule. The lowest energy conformation generated, the global minimum, was then used in a 5 nanosecond molecular dynamics simulation at 300 K to provide time averaged conformational data for comparison with collected NMR data.

5.3b Results of trisaccharide modelling

In the comparison of the ten structures obtained after the simulated annealing protocol it becomes obvious that the sequences converged to a single family of conformations (Figure 5.5). These have glycosidic torsional angles of ϕ_1 53.5°±1° ψ_1 -0.5°±2° and ϕ_2 33.5°±1° ψ_2 25.1°±4°(defined in Figure 5.1). Thus the simulated annealing protocol was successful, converging to the global minimum conformation for the given parameterization. It is interesting to note that the second glycosidic linkage adopts a significantly different combination of torsional angles than that of the first. This is likely because of steric interactions due to the vicinal substitution pattern of this oligosaccharide, which is similar in its conformational effect to vicinal branching in more rigid carbohydrate structures (such as the blood group structures). From the 5 ns molecular dynamics run for the trisaccharide it was possible to back calculate the distances expected from ROE spectra. These data were calculated based on the r^{-6} relationship between the ROE intensity and the distance between protons (Table 5.4).[192]

Table 5.4 Comparison of theoretical and experimental distances for trisaccharide **89**

ROE contacts	Trisaccharide Distances (Å)		
	Found	Calculated	Difference
Contacts across glycosidic linkage			
H2A-H1B	2.26	2.47	0.21
H2B-H1C	2.29	2.43	0.14
Contacts separated by one residue			
H4A-H1C	3.32	2.71	0.61
H4A-H2C	2.86	2.95	0.09

Oligomers are labeled alphabetically from the reducing terminus

The theoretical values and experimental distances agree when compared with data obtained for other oligosaccharides using this type of simulation.[191] Inspection of all calculated ROE distances indicated no extra contacts above those observed in the spectral data. Analysis of the conformational states explored during the dynamics simulation indicate the second glycosidic linkage spends a short period of time in the *anti*-conformation (ϕ ~180°) which has an elevated

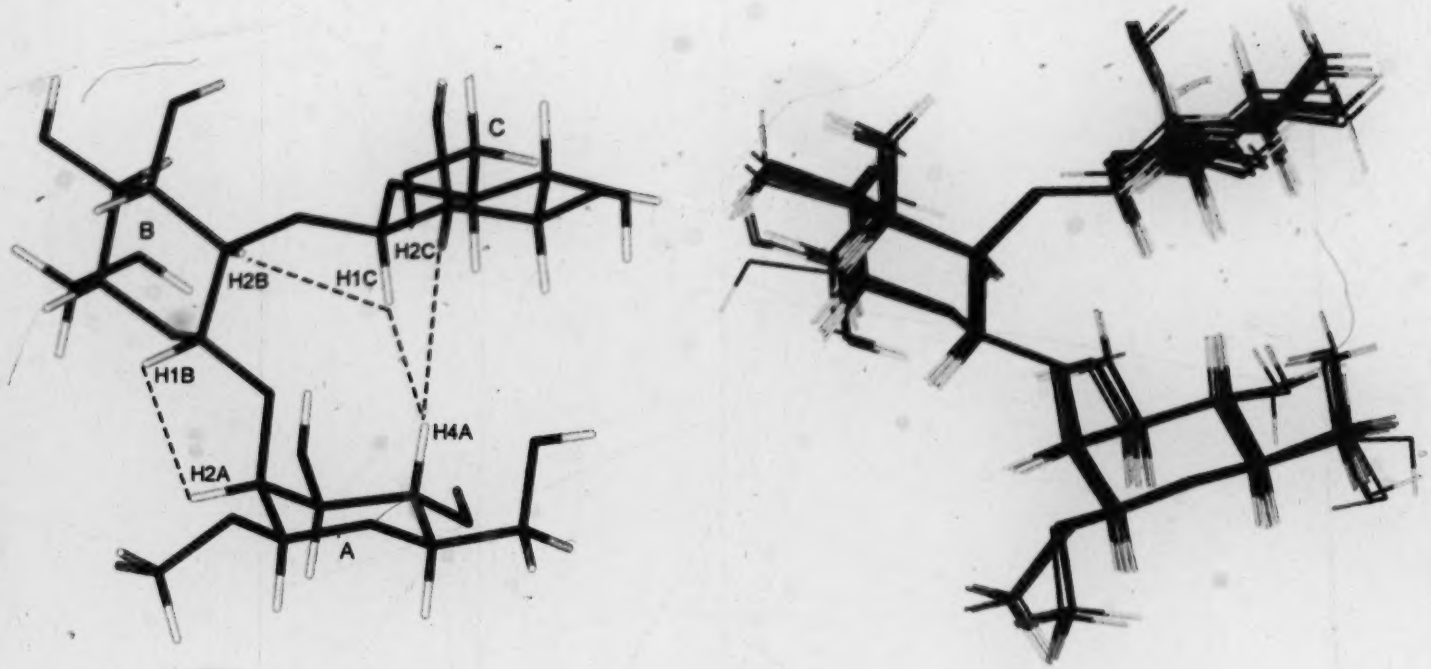


Figure 5.5. Overlay of ten structures generated by simulated annealing protocol (right). Minimum energy structure generated with inter-residue ROE contacts indicated by dashed lines (left)

energy (Figure 5.6). The observation of the structure exploring this conformation over the duration of the dynamics run indicates that the simulation samples realistic areas of conformational space and the dynamics run represents a good conformational average.

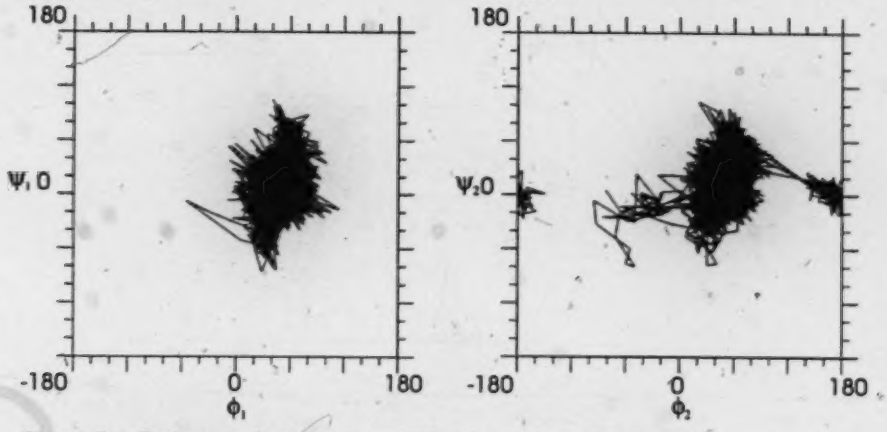


Figure 5.6. Trajectory plot of ϕ *vs* ψ during 5 nanosecond molecular dynamics run.

5.3c Results of tetrasaccharide modelling

The ten structures obtained after the simulated annealing protocol fell into three categories; one of ten contained the terminal non-reducing sugar in the *anti*-conformation (ψ ~180), two of ten contained the terminal reducing sugar in the *anti*-conformation (ψ ~180), and the remainder having the lowest energy conformations fell in a disperse group having similar torsional angles (Table 5.5).

Table 5.5.Results of simulated annealing of tetrasaccharide **90**

Structure	Relative total energy (kcal/mol)	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3
1	-4.47	54.1	-7.6	36.1	-3.0	46.7	19.2
2 (*anti*)	-3.25	**50.2**	**-176.9**	46.3	-3.1	51.6	27.6
3 (*anti*)	-1.46	44.3	-6.7	71.4	57.5	**50.3**	**-177.4**
4	-4.64	53.1	-9.5	36.9	-4.8	47.7	19.6
5	-6.45	48.2	9.6	36.5	-11.5	47.6	18.2
6	-4.90	56.1	0.4	43.4	-1.2	52.6	29.3
7	-4.83	53.6	7.7	36.6	6.9	47.3	-19.8
8	-5.74	53.1	-7.1	30.8	-5.6	47.5	20.1
9	-5.37	53.3	8.2	37.0	-7.2	47.1	20.6
10 (*anti*)	-1.88	**49.4**	**-178.7**	45.2	-5.0	53.3	28.6

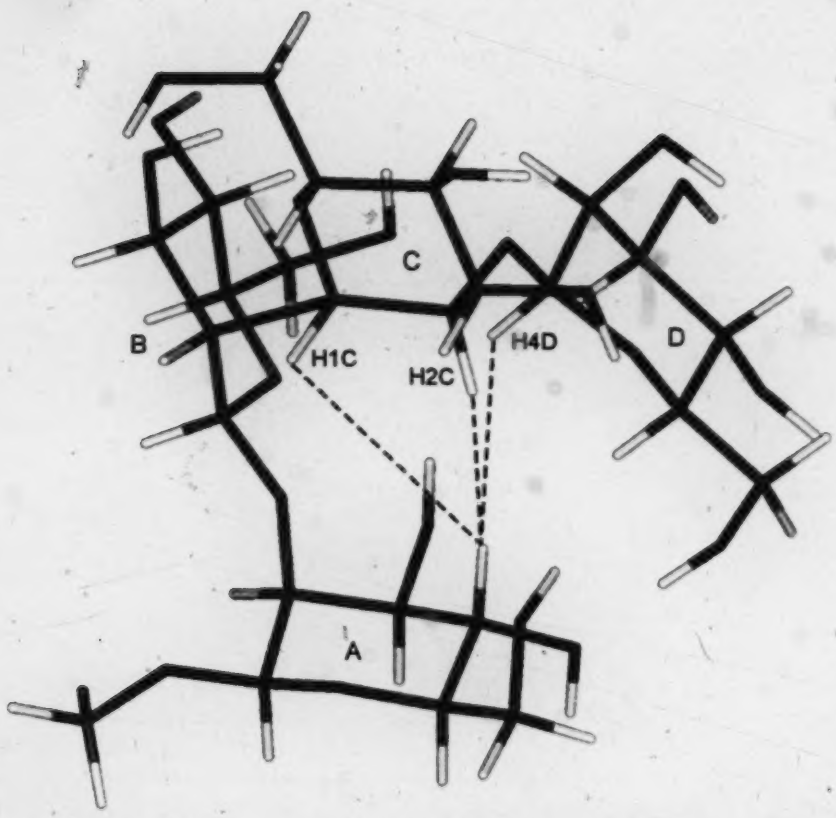


Figure 5.7. Minimum energy conformation of tetrasaccharide **90** derived from simulated annealing. Dashed lines indicate ROE contacts to the reducing terminus from non-contiguous residues.

The results of the simulated annealing protocol starting at high temperature and cooling to 5 K indicate high energy barriers to transitions between the *anti* conformations (stuctures 2,3 and 10) observed, and the population of lower energy conformers. It was not possible for terminal sugars in the high energy *anti* wells to traverse into the lower energy *syn* conformational well during the cooling phase of the simulation. Given that the majority of the conformations generated fell into the same low energy ensemble of conformations, this family is presumed to represent the global minimum conformation. The lowest energy conformation, strucuture 5, (Figure 5.7) of this family was selected and used in the 5 nanosecond molecular dynamics simulation.

Table 5.6 Comparison of theoretical and experimental ROE distances for compound **90**

ROE contacts	Tetrasaccharide Distances (Å)		
	Found	Calculated	Difference
Contacts across glycosidic linkage			
H2A-H1B	2.25	2.44	0.19
H2B-H1C	2.24	2.38	0.14
H2C-H1D	2.29	2.46	0.17
Contacts separated by one residue			
H4A-H1C	3.14	2.84	0.30
H4A-H2C	2.78	2.68	0.10
H4B-H1D	3.09	2.79	0.30
H4B-H2D	2.68	3.03	0.35
Contacts separated by two residues			
H4A-H1D	4.13	3.60	0.53

Residues are labeled alphabetically from the reducing terminus

Back calculation of the ROE distances from the dynamics simulation compared well with the experimentally determined data, containing errors on the order of 10-15%.

No large conformational changes over the duration of the molecular dynamics run performed at 300 K were observed, and similar levels of flexibility were seen for all the torsional angles. The *anti*-conformation was not sampled for either of the terminal residues, confirming the data found in the annealing simulation of high energy traverses into these conformations.

5.3d Results of pentasaccharide modelling

Further comparison of the ten structures obtained after the simulated annealing protocol showed only two families of conformations were observed. The *anti*-conformation was adopted for the glycosidic linkage between residues C and D in one of the ten conformations and was significantly higher in energy than the other nine conformations, which fell into a common family (Figure 5.9). It is interesting to note even this pentamer does not show any repetition or oscillation in torsional angles that would be expected for a homopolymer. It would likely begin to arise for the hexasaccharide, explaining the overlap observed in the ^{1}H NMR anomeric signals of residues C and D for this oligomer.

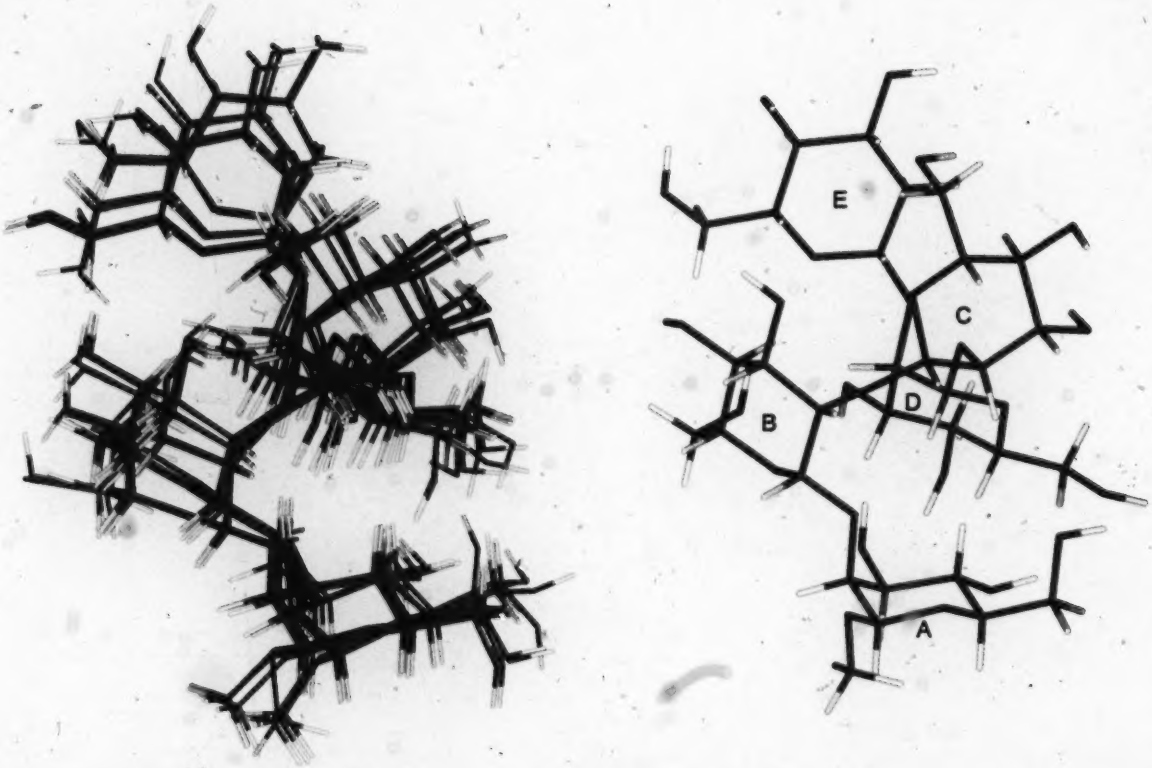


Figure 5.9. Overlay of low energy population of conformers generated by the simulated annealing protocol for pentasaccharide **91** (left). Lowest energy conformer generated in two views (right).

From the 5 ns molecular dynamics run performed at 300 K, using the minimum energy structure generated from the simulated annealing protocol as the starting structure, theoretical ROE distances were calculated. Again, the data compared well in the light of the error usually observed for this type of comparison (Table 5.7). During the dynamics run, the oligosaccharide stayed within the range of glycosidic torsional angles represented by the family generated by the simulated annealing.

Table 5.7. Comparison of theoretical and experimental ROE distances for compound **91**

ROE contacts	Pentasaccharide Distances (Å)		
	Found	**Calculated**	**Difference**
Contacts across glycosidic linkage			
H2A-H1B	2.24	2.45	0.21
H2B-H1C	2.21	2.38	0.17
H2C-H1D	Nq	2.38	-----
H2D-H1E	2.25	2.47	0.22
Contacts separated by one residue			
H4A-H1C	3.22	2.89	0.33
H4A-H2C	2.72	2.68	0.04
H4B-H1D	3.12	2.92	0.20
H4B-H2D	2.65	2.80	0.15
H4C-H1E	3.15	2.79	0.36
H4C-H2E	2.83	3.10	0.27
Contacts separated by two residues			
H4A-H1D	3.71	3.36	0.35
H4B-H1E	4.20	3.70	0.50

Residues are labeled alphabetically from the reducing terminus

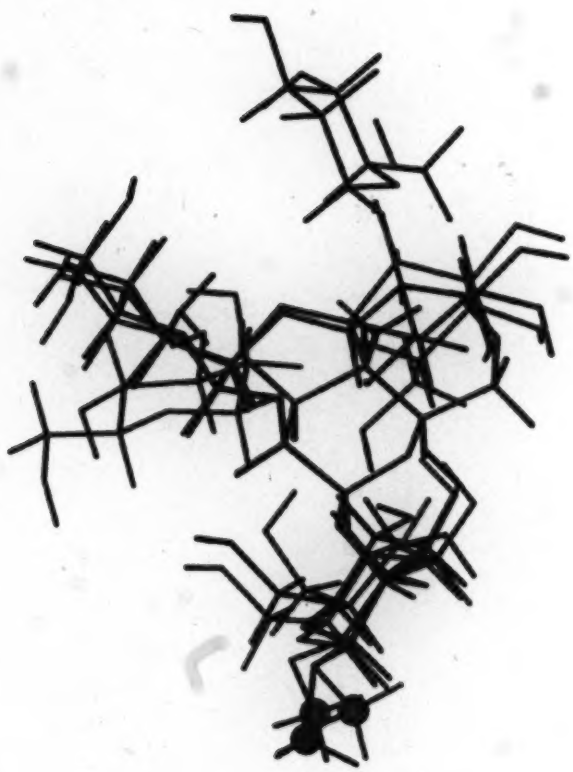
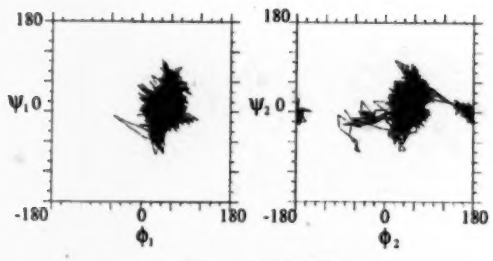


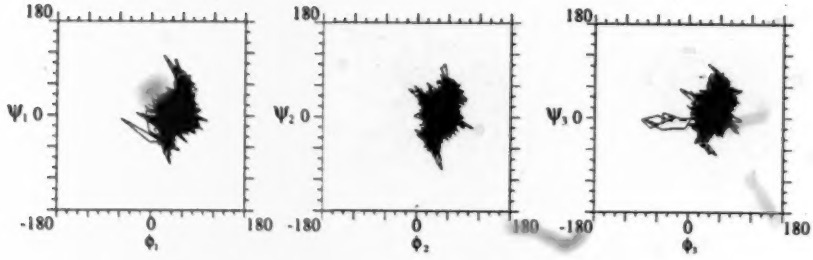
Figure 5.11. Overlay of the minimum energy conformations of the tri (green), tet (red), and pentasaccharides (blue). Ring B of each structure has been superimposed. Balls indicate the reducing terminal methyl aglycons.

5.3e Conclusions about molecular modelling

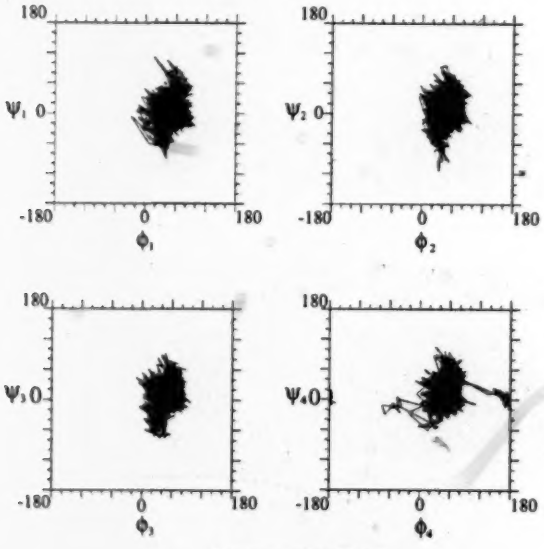
The molecular modelling of the mannopyranans succeeded in generating a tangible model of this unique oligosaccharide, which agrees well with the experimentally determined ROE intensities. Comparisons of the conformational space sampled by the oligosaccharides indicate the tri, tetra and pentasaccharides explore very similar torsional angles across all of their linkages. As expected, the molecular dynamics predicts reducing and non-reducing termini to be more flexible than the internal residues, but the experimental ROE data does not show significant increases in distances over the terminal glycosidic bonds. It is possible that despite the increased flexibility, the average distance remains the same (Figure 5.10).



Trisaccharide **89**



Tetrasaccharide **90**



Pentasaccharide **91**

Figure 5.10. ϕ *vs* ψ torsional angle trajectory maps for tri, tetra, and pentasaccharides (**89**,**90**,**91**). Definitions of torsional angle found in figure 5.1.

Comparison of the conformation of the minimum energy structures generated by the simulated annealing protocol also indicates similar torsional angles between the tri, tetra and pentasaccharides (Figure 5.11). Although the trisaccharide has its non-reducing residue tilted approximately 45° when compared with the tetra or pentasaccharides. This is an interesting result in light of the recent finding that an anti-*Candida* monoclonal antibody binds the trisaccharide but not larger oligomers.[137] It is possible that this monoclonal antibody is recognizing the trisaccharide in a conformation that is unfavorable for larger oligomers.

Given that the modelling adequately represented structures up to the pentasaccharide it was tempting to build a model of a decasaccharide to help visualize the supermolecular structure of this unique polysaccharide (Figure 5.12). This qualitative model shows the obvious helical nature of this polysaccharide. The repeating unit is approximately three residues long, because of the flexibility of the structure the overlap of residues N and N+4 is only approximate. In the helix, hydroxyls are oriented out into solution and there is hydrophobic core made up of the faces of the mannose rings. Any hypothesis about the importance of this structure to the polysaccharides function is purely speculative, but it is interesting to consider possible implications. The helix hides the glycosidic linkages at its core perhaps limiting the accessibility to endo-mannosidases. The hydrophobic faces of the rings are also shielded, except for the terminal residues, which likely has implications in the binding of the polymer by antibodies or lectins where hydrophobic surfaces are important.

5.4 Conformational analysis of the thio-tetrasacharide

5.4a Introduction

Despite the homology between the atoms, the substitution of an oxygen atom for a sulfur atom has significant implications for the overall conformation of a glycosidic linkage. The C-S bond is on average 0.4 Å longer than the C-O bond but in terms of inter-residue separation this is compensated by a smaller valence angle (C-S-C 100°, C-O-C 116°). The net

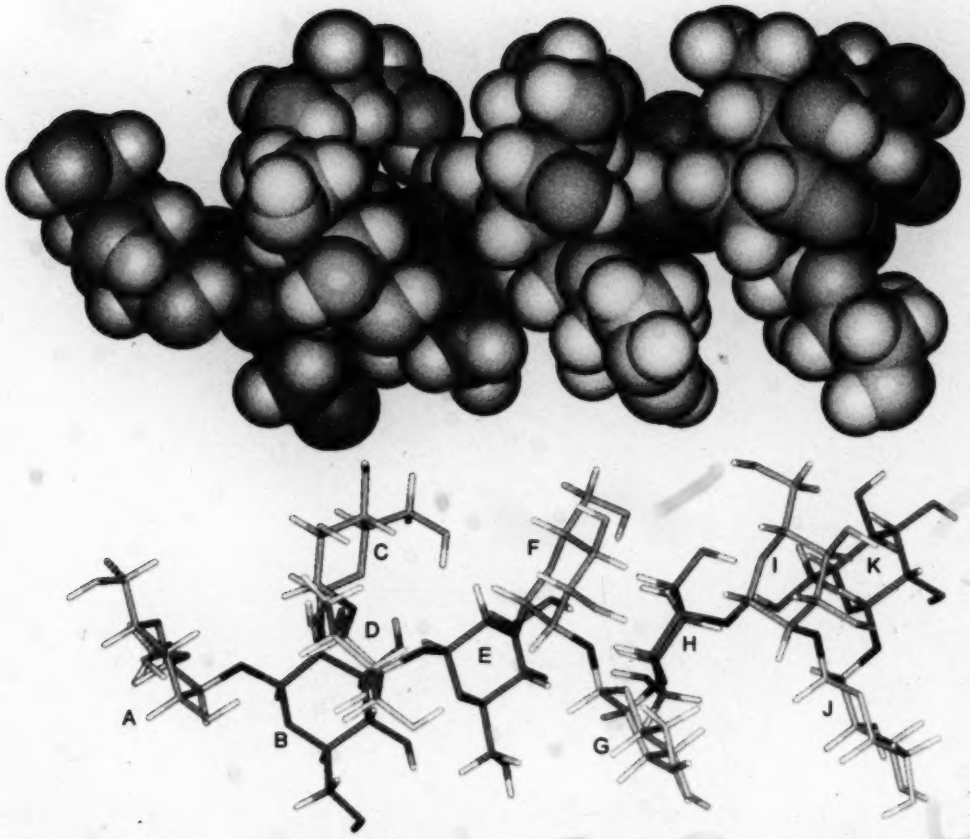


Figure 5.12 Model of a β(1→2)-mannopyranosyl undecasaccharide showing the helical nature of this polymer. The approximate trisaccharide repeating unit is emphasized by the repeating yellow, green, blue motif.

result is an increased spacing of ~0.4 Å spanning the glycosidic linkage. This reduces the steric contacts between residues generally leading to a more flexible linkage.[193] A limited number of conformational studies have been carried out on complex thioglycosides. Five disaccharides, containing a single thio-glycosidic linkage and sialyl Lewis-x mimetic containing three thioglycosidic linkages, have been analyzed with a combination of NMR and molecular modelling.[194] Unfortunately, modelling of these compounds is made difficult by the lack of parameterization of the thioglycosidic linkage in most readily available forcefields. It is well

known that thioglycoside linkages have a weaker anomeric effect than the corresponding O-glycosides. So, it is likely that the exo-anomeric effect is also weaker.[195, 196] No parameterization is available to describe this constraint thus any modelling done to this point uses parameters developed to model the thio ether without any contribution from the exo-anomeric effect. Nonetheless the MM2* force field has given excellent results for calculations of populations in low energy conformations about thioglycosides and the AMBER forcefield has been shown to give qualitative results for interpretation of the energy surface and NOE intensities for thiocellobiose,[194] and thioFuc(α1-3)GlcNAc.[194] The AMBER forcefield will be used in these studies as MM2* is not available in this laboratory.

5.4b Analysis of the ^{1}H and ^{13}C NMR spectra of propyl β(1-2) -thio-tetramannopyranoside (105)

Similar to the oxygen linked analogue, the ^{1}H NMR spectra were assigned using a combination of gradient enhanced COSY and TOCSY experiments. Again, exceptional signal dispersion was found for this compound, and all of the signals could be assigned. As would be expected the introduction of the sulfur caused upfield shifts of protons separated by two bonds, other protons close in space were also affected due to the anisotropy of the sulfur atom. Inspection of the $^{3}J_{H,H}$ coupling constants for the pyranose ring C indicated a very minimal distortion due to the presence of the sulfur atom, as $^{3}J_{2,3}$ was 4.6 Hz compared to 3.2-3.4 Hz observed for the other residues in the oligomer. The other $^{3}J_{H,H}$ coupling constants around ring C were comparable to those found in the rings of the O-linked oligomers. The magnitude of coupling constants depends not only on the dihedral angle between the atoms involved but also on the substituents present. Thus, this minor deviation may be due to the presence of a C-S bond and not a distortion of the dihedral angles of the pyranose ring.[197] No obvious perturbations were present in ring D.

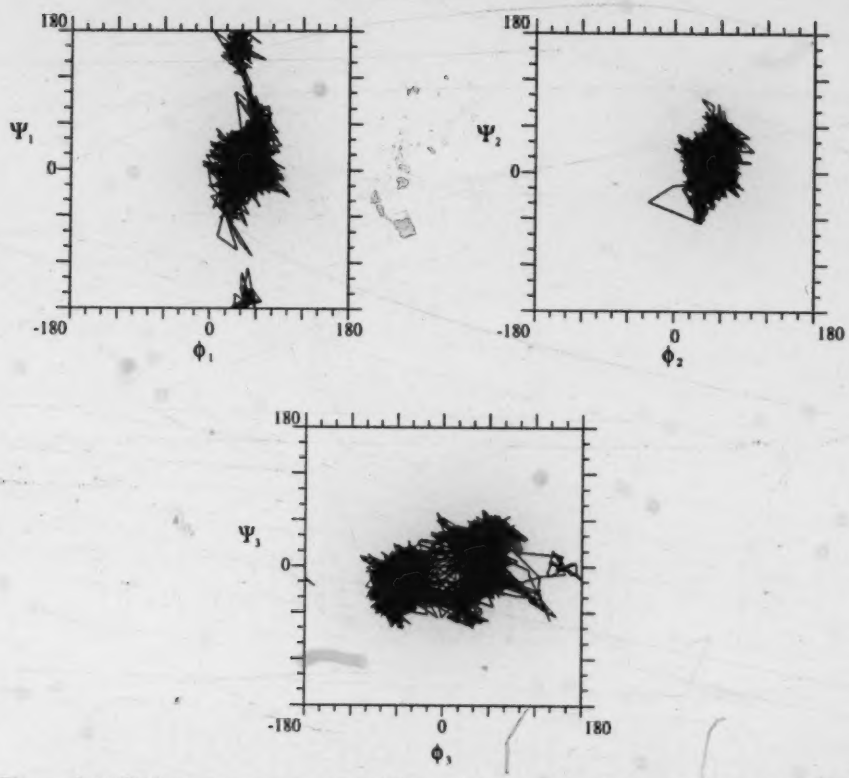


Figure 5.14 Trajectory maps generated for molecular dynamics on thioglycoside **105**

Unfortunately due to signal overlap, it was not possible to quantify as many ROE contacts in the thiotetrasaccharide (**105**) analogue as in compound **90,** its O-linked counterpart. Nevertheless, the ROE contacts present suggest a similar compact structure to that of the oxygen-linked analogues. Direct comparison between the native tetrasaccharide and its mimetic is discussed below.

5.4c Conformational analysis of propyl (1-thio-β-D-mannopyranosyl)- (1→2)-β-D-mannopyranotriose

Analysis of the ten structures generated from the simulated annealing protocol suggests a large family of conformational minima all clustered around similar torsional angles (Figure 5.13). This is expected for the thioglycoside, which should be more conformationally flexible. Surprisingly no structures contained the *anti*-conformation around the thioglycosidic linkage as has been observed for other thioglycosides.[194]

Table 5.8 Comparison of theoretical and experimental ROE distances for compound **105**

ROE contacts	Thiotetrasaccharide Distances (Å) Found	Calculated	Difference
Contacts across glycosidic linkage			
H2A-H1B	2.22	2.43	0.19
H2B-H1C	2.22	2.38	0.14
H2C-H1D	2.38	2.54	0.17
Contacts separated by one residue			
H4A-H1C	3.18	2.85	0.33
H4A-H2C	2.67	2.77	0.10
H4B-H1D	N.Q	2.72	-----
H4B-H2D	2.92	3.17	0.25
Contacts separated by two residues			
H4A-H1D	N.Q	3.85	-----

Residues are labeled alphabetically from the reducing terminus
N.Q not quantified due to signal overlap but observed

Molecular dynamics simulations of methyl (1-thio-β-D-mannopyranosyl)-(1→2)-β-D-mannopyranotriose indicate the reducing residue behaves much the same way as that of the native structures investigated above. It shows a short foray into a higher energy *anti*-conformation but returns rapidly to the preferred *syn* conformation. The second glycosidic linkage is constrained in its mobility to a small region of the energy surface encompassing the families of minimum energy conformations observed in the simulated annealing protocol. The terminal thioglycosidic linkage shows two conformations are explored during the simulation at ϕ angles centred around two gauche conformations 60° and -60° (Figure 5.14). The conformation about the thioglycosidic linkage that was observed in the simulated annealing protocol is the lower energy of the two (~60°). The exo-anomeric effect would favour the conformation at 60° and it is likely that if this were parametrized in the forcefield a greater proportion of the time would be spent in this conformation. The calculation of the ROE distances from the dynamics run agreed well with

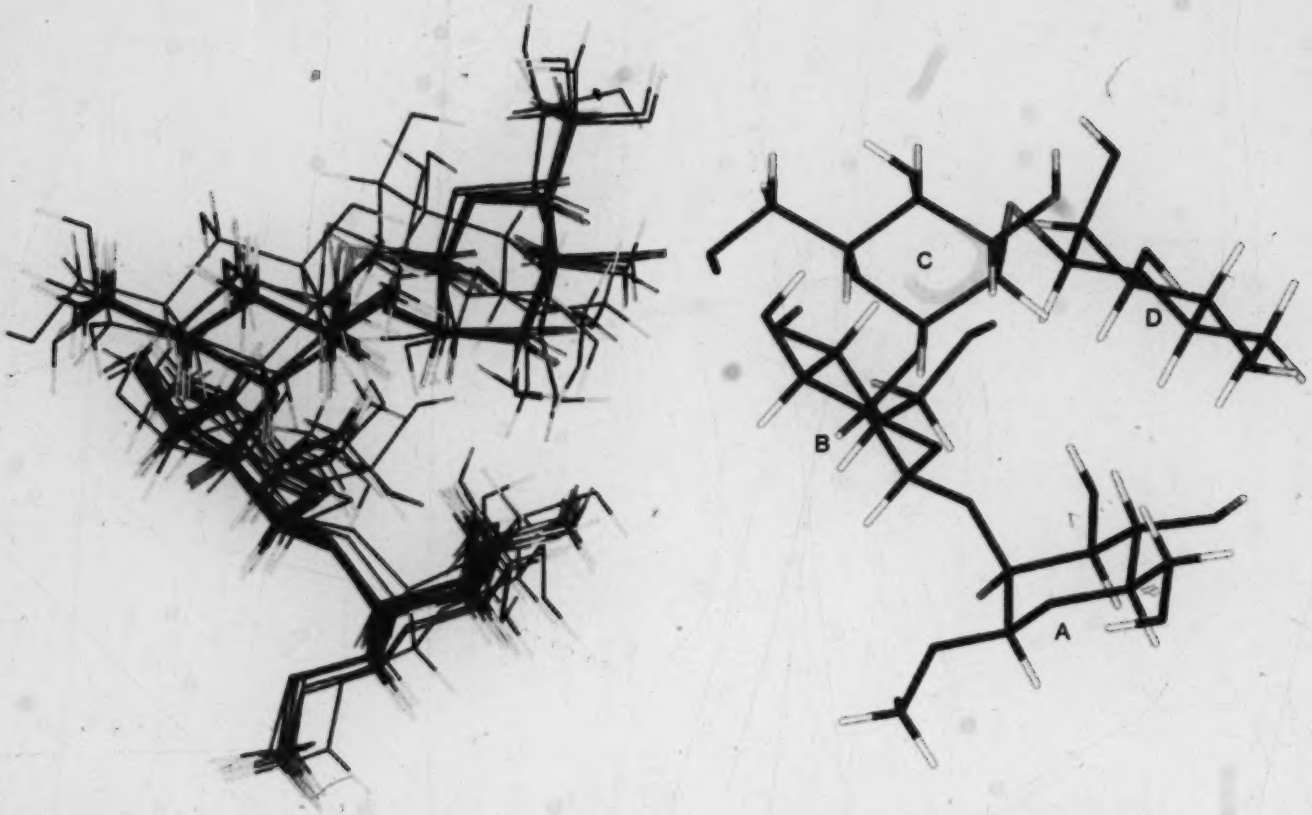


Figure 5.13. Overlay of structures generated by the simulated annealing protocol (left). Minimum energy structure (right).

those determined experimentally. The calculated H4A-H2C distance, which spans the thioglycosidic linkage, agrees well with the experimental data, suggesting in this case the AMBER forcefield performed well for modelling a thioglycoside. (Table 5.8)

5.4d Comparison of the native tetrasaccharide and the thioglycoside mimetic

Direct comparisons of the experimentally determined distances found for the thioglycoside (**105**) and the native tetrasaccharide (**90**) are very similar. Slightly longer distances are found for the distances spanning the thioglycosidic linkage (H1D-H2C, H2D-H4B) but the differences are small suggesting no gross differences between the two structures are present.

When three of the lowest energy structures for each tetrasaccharide (**90** and **105**) are superimposed it becomes obvious that both compounds sample similar conformational space but the family of low energy conformations for the thioglycoside exhibit a wider range of structures (Figure 5.15).

Table 5.9 Comparison of experimental ROE distances for compounds **90** and **105**

ROE contacts	Distances (Å) Native Tetrasaccharide 90	Tetrasaccharide mimetic 105	Difference
Contacts across glycosidic linkage			
H2A-H1B	2.25	2.22	0.03
H2B-H1C	2.24	2.22	0.02
H2C-H1D	2.29	2.38	0.09
Contacts separated by one residue			
H4A-H1C	3.18	3.14	0.04
H4A-H2C	2.67	2.78	0.10
H4B-H1D	N.Q	3.09	-----
H4B-H2D	2.92	2.68	0.24
Contacts separated by two residues			
H4A-H1D	N.Q	4.13	-----

N.Q. not quantified due to overlap but observed.

The molecular dynamics runs also suggest a more flexible structure for the thioglycoside mimetic but only about the thioglycosidic linkage. The other glycosidic linkages explore similar conformational space.

Figure 5.15 Overlay of the three minimum energy structures of the O-linked (red) and S-linked (blue) tetrasaccharides. Only backbone carbon and oxygen atoms are shown for clarity. Sulfur atom shown in yellow.

5.4e Conclusions about the conformations of the tetrasaccharide mimetic.

Like other thioglycoside mimetics previously investigated, this tetrasaccharide is more flexible than its native counterpart. However it is less flexible than the other thioglycosides that have been analyzed, as is conspicuous by the absence of any *anti*-conformation about the thioglycosidic linkage during the molecular dynamics simulation. Thus, this tetrasaccharide mimetic will suffer from a greater entropy loss when it is bound in a single conformation by a protein than the native structure, but this loss of affinity may not be as large as that observed for other thioglycoside mimetics which explore a greater variety of conformations.[194]

Chapter 6

Developments towards a Synthetic anti-*Candida albicans* Carbohydrate based Vaccine

6.1 Introduction

6.1a Background

Carbohydrates expressed on the surface of bacteria were recognized as protective antigens for immunotherapy many years ago.[198] Increasing resistance of bacteria to antibiotics has brought renewed attention to the development of carbohydrate based vaccines against a broad array of common bacterial pathogens. Carbohydrates found on the surface bacterial cells are specifically attractive vaccine targets because, as secondary gene products, they are highly conserved and, unlike proteins, no mutation of bacterial polysaccharides has thus far been reported. [199] Recently cancers have also become targets for vaccines based on carbohydrates. Cancer cells often express aberrant glycosylation patterns on their surface and these foreign antigens are being used as the basis for carbohydrate vaccines.[200]

Carbohydrates are not good antigens when used alone, regardless of their size. This is due to the way in which the mammalian immune system processes antigens to produce an antibody response. The antigen processing pathway is a very complicated process which has been studied in detail at the genetic and structural level.[201] As a brief introduction it is possible to classify

antibody-based immune responses into two categories: thymus independent (not requiring T-helper cells) and thymus dependent (requiring T-helper cells) responses (Figure 6.1).

In a T-helper cell independent response the B-cells produce an IgM antibody due to crosslinking by an antigen of their cell surface immunoglobulin receptors. This type of response is seen with polysaccharide antigens. IgM antibodies have inherently low affinity for the antigen and there is no immunological memory associated with this type of response. (Figure 6.1)

In a T helper cell dependent response the B-cells bind the antigen on their surface immunoglobulin and internalize the antigen immunoglobulin complex. The antigen is processed into small fragments, which are loaded into a large transmembrane protein called a major histocompatibility complex (MHC). This complex is transported to the surface of the cell and is then recognized by a T-helper cell through its T-cell receptor. This is a very specific recognition process, hinging upon the recognition of the antigen fragment by the MHC and the T-cell receptor, as well as recognition of the MHC by the T-cell receptor. The T-helper cell then sends a signal to the B-cell causing proliferation of this cell. At this time the B-cell stops producing IgM antibodies and begins producing IgG antibodies, a process known as an isotype switch. The rapidly proliferating B-cells produce mutations in their IgG antibodies and those cells with increased affinity are selected in a process known as affinity maturation. These selected cells mature into IgG antibody secreting plasma cells or memory cells. Thus the plasma cells secrete high affinity IgG antibody, and the memory cells can be quickly activated in subsequent encounters with the same immunogen to produce high affinity antibodies (Figure 6.1).

A successful vaccine should activate a thymus dependent response, to ensure cellular memory and high affinity antibodies. A thymus dependent response is only possible with protein containing antigens because the MHC complex can present only peptide fragments. Thus, oligosaccharides alone are non-immunogenic or like polysaccharides are at best thymus independent antigens.

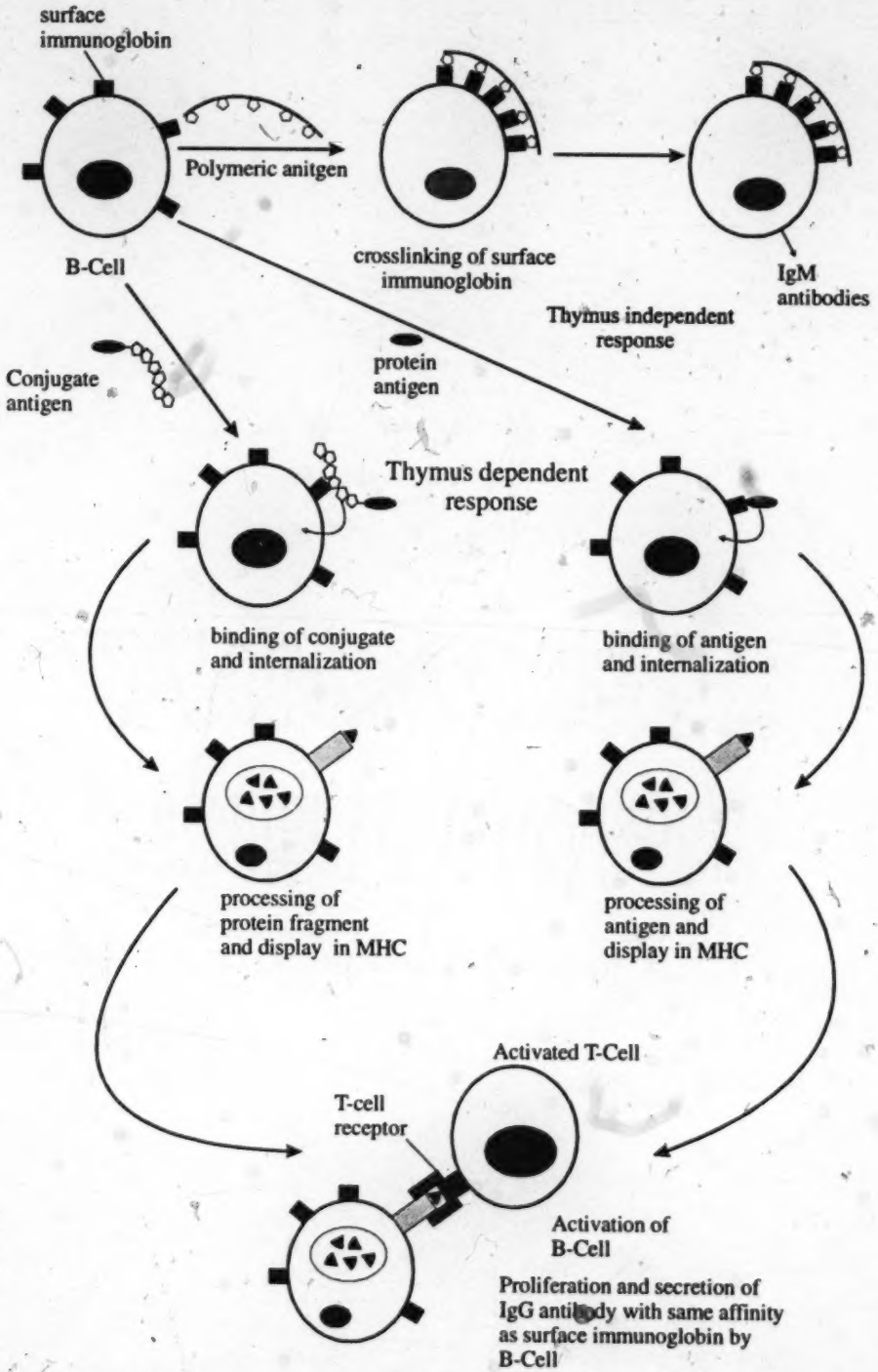


Figure 6.1. B-cell responses to antigens

It is possible to generate a thymus dependent response to carbohydrates by linking them covalently to protein carriers. Thus the B-cell can recognize the carbohydrate with its cell surface immunoglobulin, internalize the antigen, and process the protein component as it would for a protein antigen. This process leads to plasma cells and memory cells with high affinity for a carbohydrate epitope (Figure 6.1).

Using this approach many carbohydrate based vaccines have been developed and tested in experimental animals.[199] A well characterized study comparing an isolated polysaccharide and four different synthetic oligosaccharides conjugated to human serum albumin showed in mice that the synthetic oligosaccharides gave stronger antibody responses than the isolated polysaccharide conjugates.[202] Conjugates of oligosaccharide cancer epitopes and proteins have also been synthesized by numerous groups, and recently human trials have begun for: Lewis-y conjugated to keyhole limpet hemocyanin (KLH) against ovarian cancer[203], globo-H-KLH against prostate and breast cancers[200] and Tn and TF antigens linked to KLH for prostate cancer.[204]

6.1b Development of an anti *Candida albicans* vaccine

It has been shown that linking crude cell wall extracts of *C. albicans* polysaccharides to a protein will produce a vaccine that induces a protective antibody response.[136] Analysis of a monoclonal antibody produced against this crude polysaccharide vaccine showed that the recognized epitope was a trisaccharide, (1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man, which is found in the acid labile portion of the cell wall.[137] Although this information is crucial for the development of a vaccine, naturally isolated polysaccharide vaccines are plagued by problems such as heterogeneity, and the potential for biological contamination, which limit their use in humans. Synthetic carbohydrate vaccines are well defined and overcome these problems.

Three factors should be considered when developing a synthetic carbohydrate based vaccine: the size of the carbohydrate, the conjugation chemistry, and the choice of the carrier protein.

The size of the carbohydrate should provide a balance between the ease of synthesis, and how well it will mimic the natural structure. In the case of bacterial polysaccharides of Chlamydia, which contain the pentasaccharide repeating unit α-Kdo*p*-(2→8)-α-Kdo*p*-(2→4)-α-Kdo*p*-(2→6)-β-D-GlcNAc*p*-(1→6)-β-D-GlcNAc*p*-(1→, it was found that a tetrasaccharide (lacking one α-D-GlcNAc) or the complete pentasaccharide repeating unit was able to generate antibodies which were able to recognize the natural lipopolysaccharide.[205] Thus, despite the fact that the antibody binding site is usually filled by 3 to 4 pyranose rings of a carbohydrate epitope, it is necessary to have a larger structure to order these contact residues as they would be in the natural structure.[206]

The (1→2)-β-D-mannan found in the cell wall of *Candida albicans* is a homopolymer and at the time of the synthesis it was difficult to speculate as to the repeating secondary structure of this polymer. It was decided that a hexasaccharide and a tetrasaccharide would be synthesized to accurately represent the repeating nature of this polymer. Given the unexpected findings of the conformational analysis reported in the previous chapter, the trisaccharide is the minimum conformational repeating unit, and in order to have the correct conformation of the repeating unit, it is likely that a hexasaccharide is a good representation of the minimal structure necessary to represent the (1→2)-β-D-mannan.

There are many ways which have been employed to link the oligosaccharide to the protein carrier.[207] With isolated polysaccharides often the free aldehyde of the reducing terminus is used in a reductive amination reaction with the amines found on the surface of the carrier protein.[208] This destroys the reducing terminal sugar which is not a major problem with high molecular weight polysaccharides, but is not an option with smaller synthetic oligosaccharides. A terminal aldehyde has been introduced in different ways to synthetic oligosaccharides. An allyl group can be subjected to ozonolysis, or a protected aldehyde in the form of an acetal can be incorporated late in the synthesis.[209] Unfortunately reductive amination can be an inefficient

reaction when small amounts of an expensive synthesized carbohydrate are coupled. Other methods have also been introduced such as the introduction of a thiocyanate, via reaction of a *p*-aminophenyl group with thiophosgene followed by in situ coupling to a protein.[210] The use of an acyl azide, formed from reaction of a hydrazide with nitrous acid, allows rapid coupling to lysine amino groups.[211] Thioether chemistry has also been used via introduction of a terminal thiol to the oligosaccharide followed by reaction with a protein onto which a bromoacetate has been installed by reaction with α-bromoacetyl succinimide ester.[212] Perhaps the most efficient protocol that has been developed involves the use of the homo-bifunctional reagent diethyl squarate.[213] Sequential reactions of this reagent with two amines allow reproducible coupling in high yield and efficiency under mild conditions, with small amounts of oligosaccharide and protein.[214] This was the method chosen for the formation of the glycoconjugates in this study.

The choice of protein as a carrier is much less systematic. A protein carrier should be highly soluble, possess numerous functional groups for conjugation, and not be native to the animal to be immunized. No consensus has been reached as to the most efficient carrier for generating an immune response.[215] In mice a variety of proteins have been used such as bovine serum albumin (BSA), human serum albumin (HSA), tetanus toxoid (TT), keyhole limpit hemocyanin (KLH), as well as short peptides which do not require processing for display in the MHC complex.[216] In this study tetanus toxoid was chosen as a carrier because it has shown good immune responses in rabbits and mice. It is also well tolerated in humans as it is already used as a vaccine against tetanus, and, via a collaboration, it was readily available in this laboratory.

6.2 *C. albicans* conjugate vaccines

6.2a Synthesis of protein conjugates

Reaction of the mannopyranoside functionalized glycosides with diethyl squarate in a solution of ethanol and water provided, in high yield, the activated oligosaccharides ready for coupling to protein. (Figure 5.2)

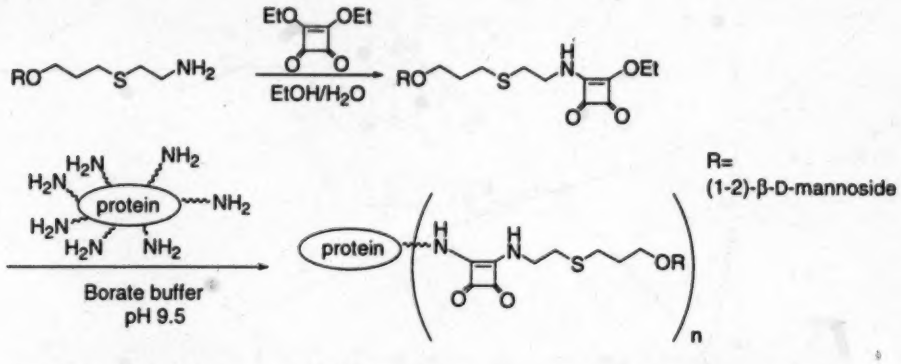


Figure 6.2. Synthesis of protein conjugates

Coupling to tetanus toxoid (TT) or bovine serum albumin was achieved using a procedure similar to that developed by Hindsgaul *et al.*[214] Coupling efficiencies were similar to those published for the oligosaccharides to BSA with efficiencies between 65-70%. This corresponds to the incorporation of 13-15 ligands with a 20 fold molar excess of activated oligosaccharide. Lower efficiencies were obtained for coupling to tetanus toxoid, as higher incorporations were desired. Efficiencies in the range of ~35% were achieved in these reactions. It is possible that the majority of the 106 amine groups of TT were buried in areas of the protein inaccessible to the coupling reagent. No quantitative data is available in the literature for coupling to tenanus toxoid, only estimates of carbohydrate loading generated by size exclusion chromatography have been used in the past.[217] Targeted and found incorporations are tabulated below (Table 6.1). The molecular weights of the protein conjugates were determined by MALDI-TOF spectroscopy. All the conjugates produced had limited polydispersities corresponding between one and two conjugations. (1→2)(-α-D-glucopyranosyl)(1→2)-β-D-mannopyranotriose

was also conjugated to BSA to serve as a control compound to test the specificity of the immune response.

Table 6.1. Protein carbohydrate conjugates

Oligosaccharide	Target incorporation (100% conversion)	Incorporation achieved	Incorporation efficiency
M_2-BSA	20	16	80
M_3-BSA	20	15	75
CM_3-BSA	20	15	75
M_4-BSA	20	13	65
M_6-BSA	20	10	50
SM_4-BSA	20	15	75
SM_4-TT	80	33	41
M_4-TT	80	27	34
M_6-TT	80	29	36

M_3 (1→2)-β-D-mannopyranotriose; CM_3 (1→2)(α-D-glucopyranosyl)(1→2)-β-D-mannopyranotriose, M_4 (1→2)-β-D-mannopyranotetrose, SM_4 (1→2)(1-thio-β-D-mannopyranosyl)(1→2)-β-D-mannopyranotriose, M_6 (1-2)-β-D-mannopyranohexose

6.3 Immunization of experimental animals

New Zealand white rabbits and inbred BALB/c mice were immunized with the tetanus toxoid conjugates as outlined in Chapter 8. In previous studies these strains of rabbit and mice have shown strong immune responses against polysaccharides conjugated to tetanus toxoid.[218,219]

6.3a Analysis of antibody levels

The titers of antibodies produced in the experimental animals were analyzed by ELISA. The antigen to be tested was adsorbed to 96 well ELISA plate and the plate was washed. To the wells were added serial dilutions of the sera from the immunized animal to be analyzed. After incubation of the plate unbound antibodies were washed from the plate. A horse radish peroxidase conjugated antibody against the antibody to be analyzed was used to quantify the amount of specific antibody bound to the plate.

6.3b Antibody levels in experimental animals

Response in mice

The mice developed a strong IgM response against the hexasaccharide tetanus toxoid conjugate after two immunizations, as judged by ELISA assay, using the BSA conjugated hexasaccharide. Weak IgG antibodies were also present after this time. Two mice were sacrificed after a third boosting immunization and using hybridoma technology attempts to isolate a monoclonal antibody were carried out. Unfortunately only one monoclonal antibody was selected and it had very weak titers against the *C. albicans* antigens. Surprisingly only weak IgM and IgG antibodies could be raised with a subsequent booster injection. The possibility that the mice had only raised antibody against the carrier was analyzed by substituting the carrier protein for the BSA conjugate in the ELISA assay. No antibodies against the carrier protein were found. This is a highly unusual and suspect result given the good results found previously with this carrier. Future work will examine the immunization of mice with similar conjugates as well as immunologically more potent carriers, such as bacterial porin proteins.

Response in rabbits

Rabbits showed a strong IgG response after three injections with the tetanus toxoid conjugates. In order to better quantify the specificity of this polycolonal response, titrations of the antibody against all the BSA conjugated oligosaccharides were undertaken. Surprisingly, it was found that the polyclonal sera recognized all the conjugates with similar affinity, even the control compound terminated by a α-(1→2)-D-glucopyranosyl residue was recognized. It was originally envisaged that oligosaccharides conjugated to the heterologous protein, BSA, would distinguish between antibodies that where directed toward the carrier protein of the immunizing antigen and those against the desired oligosaccharide. However, since squarate coupling chemistry was common to the TT and BSA conjugates, antibodies that recognize this structurally prominent feature may be detected in the assay. If antibodies against the linker are being detected

in the assay this would explain why all the BSA glycoconjugates appear to have the same affinity for the rabbit sera. This hypothesis will be explored further by the synthesis of a glucopyranoside glycoconjugate containing the same linking chemistry as the mannosides. If the rabbit sera recognizes this structure it will indicate the immune response was biased towards the linker and that in the future other linking chemistries may be more appropriate for use in the generation of synthetic carbohydrate based vaccines.

Based on different titers against neoglycoconjugates M_3-BSA and CM_3-BSA it was evident that a significant fraction of the antibodies did not bind the control compound. This suggested that there likely were antibodies present that were specific for the (1→2)-β-mannosyl oligosaccharide. This possibility was analyzed by screening the sera from these rabbits against cell wall extracts of *C. albicans*. These assays showed a high titer of IgG antibody that recognizes the native *C.albicans* antigen (Figure 6.3). Both the hexa and tetrasaccharide TT conjugates raised similar antibody titers. Further investigations on the ability of these antibodies to provide passive protection against challenge with *C. albicans* in mice are on going in Professor Jim Cutler's laboratory at the Montana State University.

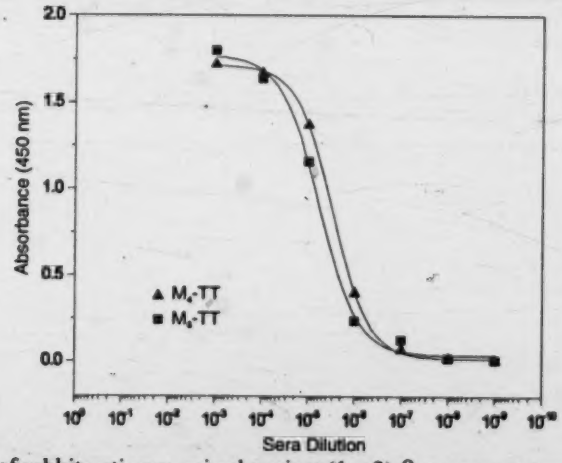


Figure 6.3 Titration of rabbit anti sera, raised against (1→2)-β-D-mannotetrose (M_4-TT) and (1→2)-β-D-mannohexaose (M_6-TT) tetanus toxoid conjugates, with *C. albicans* mannan antigen coated on ELISA plates

6.3c Binding of propyl (1→2)-β-mannosides to monoclonal antibodies

Isolation of IgM and IgG monoclonal antibodies, generated from immunizations of mice with liposomal extracts of the *C. albicans* cell wall, have been carried out in Professor Jim Cutler's laboratory.[135] These monoclonal antibodies have been shown to be protective against *C. albicans* challenges in passive transfer experiments.

The affinity of these monoclonal antibodies to the synthesized propyl glycosides was determined in a competitive ELISA, which is similar to the assay shown in Figure 2.11. In this application of the assay a *C. albicans* mannan extract was used to coat ELISA plates. The results from this assay are shown as a percentage inhibition, and the concentration of ligand that gives 50% inhibition is quoted as an IC_{50} value. The lower this value, the higher the affinity of the ligand for the antibody being studied.

An IgM antibody shows a surprisingly high affinity for the di and trisaccharides when compared to the tetra and hexasaccharides. The propyl (1-thio-β-D-mannopyranosyl)-(1→2)-β-D-mannopyranotrioside falls between the tri and tetrasaccharide in affinity (Figure 6.4).

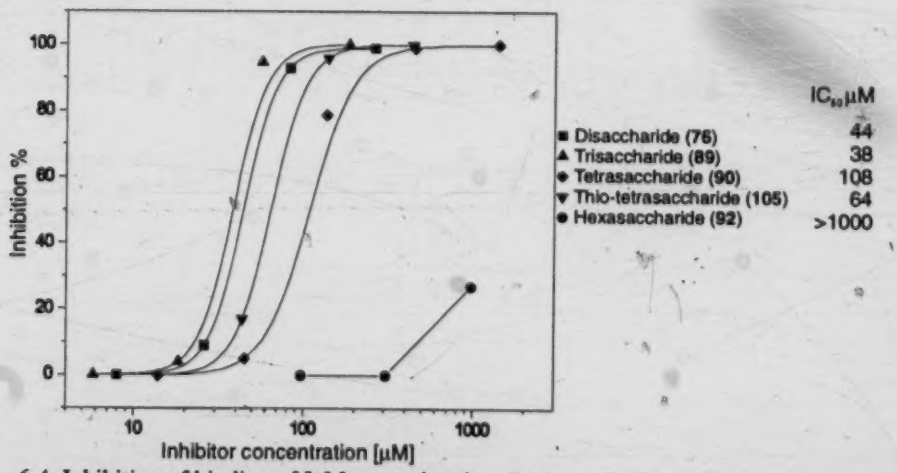


Figure 6.4. Inhibition of binding of IgM monoclonal antibody to *C.albicans* mannan extract with propyl mannosides (**76**, **89**, **90**, **105**, **92**)

The IgG antibody showed a similar trend of affinities, to that of the IgM antibody, for the same panel of oligo mannosides. The di and trisaccharides had higher affinities, five and two fold

respectively, for the IgG antibody than the IgM MAb and the remaining antigens had a similar affinity (Figure 6.5).

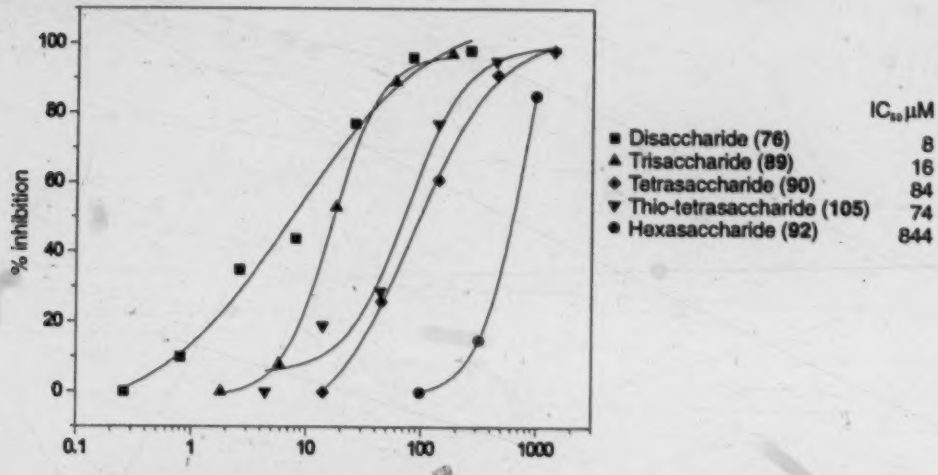


Figure 6.5 Inhibition of binding of IgG monoclonal antibody to C. albicans mannan extract with propyl mannosides (**76**, **89**, **90**, **105**, **92**). The origin of the flattened the binding curve for the disaccharide is unknown

To the best of our knowledge the observation of higher affinity for smaller oligomers is unprecedented for antibodies generated to polysaccharide antigens. As a rule the larger oligomers have higher affinity for the antibodies. Beginning with the pioneering studies of Kabat in which he immunized himself with dextran ((1→6)-α-D-glucose) the expected trend is to observe oligomer inhibition that increases with oligosaccharide length to a maxium of 5-8 hexose residues.[206] At a certain size, which may vary with the precise pool of antibodies, the inhibition plateaus when expressed on a molar basis. To date rather unique inhibition patterns have been reported for homo-oligomers of sialic acid but the increased inhibitory potency correlated with oligomers even longer than 8 residues, in this case reaching a 20-mer.[220]

Furthermore, it is very surprising that the IgG and IgM antibodies have very similar specificity and affinity. Statistically one would expect, using a polydisperse antigen such as the extract used to generate these antibodies, antibodies with differing epitopes would be generated.

The finding that both monoclonal antibodies recognize similar epitopes suggests a single immunodominant epitope in the extract of the *C. albicans* cell wall used for immunization.

As these antibodies are known to be protective, the inhibition results indicate a near ideal situation for the generation of synthetic vaccines. The major criticism of synthetic vaccines is that it is not economical to synthesize the size of epitope necessary to generate a protective immune response (15-20 hexose residues).[19] If only a disaccharide or trisaccharide epitope is required for conjugation this caveat is unfounded.

Examination of the conformation of the oligosaccharides and a proposed binding mode for the mannopyrans may explain the unexpectedly high affinity of the di and trisaccharides for the monoclonal antibodies. It appears a terminal disaccharide is the epitope for the monoclonal antibodies. The elaboration to the trisaccharide gives an inhibitor of similar affinity but further elaboration of the polymer leads to reductions in affinity. Due to the helical nature of this polymer the fourth mannopyranose ring (residue D) comes in close proximity to the reducing residue (residue A). Residue D may then be causing steric interactions with the antigen binding site or a part of the antibody surface adjacent to the site (Figure 6.6). It has previously been observed that hexoses outside the binding site of an antibody can adopt higher energy conformations to avoid steric interactions with the protein.[221]

The nature of this steric interaction can explain the order of the affinity observed for the tetra (**90**), thio-linked tetra (**105**) and hexasaccharides (**92**). The terminal thioglycoside of the propyl (1-thio-β-D-mannopyranosyl)-(1→2)-β-D-mannopyranotriose (**105**), has the most flexible terminal glucosidic linkage (C→D) when compared with **90** and **92**. Thus, residue D is able to adopt another low energy conformation about this terminal linkage to avoid unfavorable steric interactions with the protein. The tetrasaccharide, which binds with intermediate affinity, has a less flexible fourth residue (D) but it is still able to minimize the steric interactions with the protein by adopting another conformation. Finally the hexasaccharide, which surprisingly binds

with low affinity, has the most rigid fourth residue (D) due to restricted mobility about the C→D glycosidic linkage enforced by the subsequent residues of the helix. It cannot easily adopt another conformation leading to unresolved steric interactions with the protein and a low affinity inhibitor.

If this hypothesis is correct it suggests that the antibody is recognizing the reducing terminus of the polysaccharide. This finding has implications for the *in vivo* activity of the antibody, as the reducing terminal structures may be inaccessible because of their linkage to the remainder of the mannan making up the cell wall.

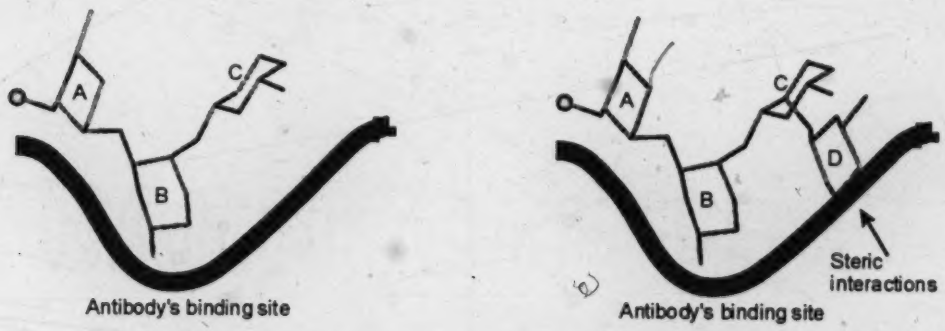


Figure 6.6. Possible steric interactions in the antibody binding site disfavoring binding of epitopes larger than trisaccharide. Only backbone carbon and oxygen atoms have been included for clarity. The ball indicates reducing terminal aglycon.

This raises the question as to what native epitope induced the formation of monoclonal antibodies, especially since the monoclonal antibodies show *in vivo* protection. Short sequences of (1→2)-β-D-mannopyranan do exist in the phosphomannan of the yeast, although trisaccharide and longer polymers generally predominate.[222] If the native epitope is a short sequence from the phosphomannan, it is likely that the antibody accommodates a negative charge from the phosphodiester in the native antigen that links the reducing terminus to the α-mannan backbone. Inhibitors with this type of structure may bind more tightly to the antibody.

Other (1→2)-β-D-mannopyranose linkages have been found in yeast cell walls. Suzuki *et al.* has proposed that some of the (1→2)-α-D-mannan branches are capped by a (1→2)-β-D-

mannobiose residue in serotype A. strains of *C.albicans*.[222] It is possible that an undetermined structure similar to this could be present in serotype B strains, used to generate the monoclonal antibodies, and be the native epitope for these monoclonal antibodies. But, this seems unlikely given that Cutler *et al.* did not find the monoclonal antibodies bound the acid stable portion of the *C. albicans* cell wall, where these structures are proposed to occur.[137]

6.4 Conclusions

Further studies are needed on the potential of using the conjugates generated in these studies as anti *Candida albicans* vaccines. It is very surprising that in the mouse studies no IgG antibodies against the conjugate or the carrier protein alone were generated. Numerous studies have successfully used tetanus toxoid for the generation of carbohydrate conjugate vaccines with the same mouse strain. (219 and references cited therein)

A strong antibody response was generated in the rabbit against the tetanus toxoid conjugates and these antibodies recognize the *C. albicans* extract. Further studies are needed to determine the level of protection these antibodies confer.

Two monoclonal antibodies (IgM and IgG) previously isolated in another laboratory have been shown to have higher affinity for smaller (di and trisaccharides) over larger structures (tetra and hexasaccharides). This unexpected result has been rationalized with the compact helical nature of the (1→2)-β-mannan polymer and potential steric interactions with the binding site. This finding suggests a synthetically simple disaccharide or trisaccharide conjugate may be all that is necessary to generate a protective immune response.

Further studies are on going to isolate a monoclonal antibody to the synthetic neoglycoconjugates synthesized here. It will be interesting to determine if the specificity of these antibodies is similar to those raised against the natural structure and if the level of protection they confer is similar.

Chapter 7

Conclusions and speculations about *Candida albicans* (1→2)-β-D-mannopyranans

The synthesis of these unique polymers required novel modifications of an existing procedure for the synthesis of β-D-mannopyranosides. The modulation of the glycosyl donors reactivity, by the introduction of *p*-chlorobenzyl protecting groups, was required to stabilize the ulosyl bromide to the innovative reaction conditions. These conditions employed a sterically hindered participating solvent and a soluble to promoter to control the stereochemistry of the glycosylation, providing synthetic access to the interesting (1→2)-β-D-mannopyranans.

The *p*-chlorobenzyl protected ulosyl bromide glycosyl donor also provided access to a thioglycoside mimetic of (1→2)-β-D-mannotetrose. This compound proved to be a useful mimetic to distinguish between different binding modes of monoclonal antibodies to the (1→2)-β-D-mannopyranan oligosaccharide. The thioglycoside analogue will be used in the future to provide antigens that are resistant to enzymatic degradation yet mimic natural structures when presented to the immune system.

The conformational analysis of these unique polysaccharides with the use of NMR and molecular modelling has shown the structures to exist as exceptionally compact helices with an approximate trisaccharide repeating unit. It can be speculated that this structure is important to the biological role of the oligosaccharide. The helix may shield its glycosidic linkages from the action of endoglycosidases stabilizing the structure. This conformation may also be limiting the access to the hydrophobic faces of the mannose residues making the structure difficult for binding proteins to address.

Preliminary investigations using the synthetic (1→2)-β-D-mannan conjugates as vaccines are promising. In rabbits a strong antibody response was raised and a significant portion of these antibodies recognize the cell wall mannan extracts of *C. albicans*. Further work on the level of protection these antibodies confer are in progress as a collaboration with Professor Jim Cutler at the Montana State University.

Inhibition studies of monoclonal antibodies, previously raised against *C.albicans* extracts and shown to be protective against *C. albicans*, gave the unique result of recognizing (1→2)-β-D-manno-disaccharide and trisaccharide with higher affinity than the higher molecular weight oligomers. The molecular modelling studies have shown that the trisaccharide has a similar conformation to the higher molecular weight compounds but it is likely, given the compact helical nature of the oligomer, that introduction of the fourth and subsequent residues leads to steric interactions outside the binding site of the antibody that reduces the affinity for larger oligosaccharides. Future work may use these results to develop a synthetic vaccine containing disaccharide or trisaccharide conjugates which would constitute a synthetically tractable objective which is not the case for the larger oligomers developed here.

Although only two monoclonal antibodies are available against the *C. albicans* polysaccharide it is tempting to speculate why these both recognize smaller saccharides over the larger structures. This may be an evolutionary adaptation by the yeast. Perhaps the mice lack

the immune repertoire to recognize these larger structures because antibodies against the larger oligomers cross react with an endogenous structure. Thus, the yeast can mask themselves from the immune system using the (1→2)-β-D-mannans. This hypothesis may explain why it was not possible to raise antibodies in mice with the tetra and hexasaccharide tetanus toxoid conjugates.

Recently the finding that mammalian galectin-3 binds to (1→2)-β-D-mannan has suggested other possible biological roles for these structures.[223] Galectin-3 is expressed by a wide variety of epithelial and inflammatory cells. Thus, (1→2)-β-D-mannan may be involved in the adherence mechanisms of the yeast. More interestingly, it may be involved in the suppression of the immune response to the yeast by preventing activation of the inflammatory response thought to occur by crosslinking of cells mediated by galectin-3. Collaborations with Professor Fu-Tong Liu at the La Jolla Institute for Allergy and Immunology are focused towards exploring these possible roles of the oligosaccharide.

Chapter 8

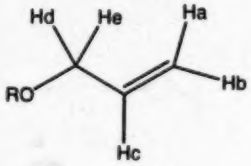
Experimental

General methods

Analytical thin layer chromatography (TLC) was performed on silica gel 60-F_{254} (Merck). TLC detection was achieved by charring with 5% sulphuric acid in ethanol. All commercial reagents were used as supplied. Column chromatography used silica gel (SiliCycle) and solvents were distilled. High performance liquid chromatography (HPLC) was performed using a Waters HPLC system which consisted of a Waters 600S controller, 626 pump, and 486 tunable absorbance detector. HPLC separations were performed on a Beckmann C_{18} semi-preparative reversed-phase column with methanol and water as eluents. ^1H NMR spectra were recorded at either 300, 360, 500, 600 or 800 MHz, and are referenced to internal standards of the residual protonated solvent peaks; δ_H 7.24 ppm for solutions in $CDCl_3$, δ_H 4.78 ppm for solutions in CD_3OD or 0.1% external acetone (δ_H 2.225 ppm) for solutions in D_2O. ^{13}C NMR spectra were recorded at 150 MHz and are referenced to internal $CDCl_3$ (δ_C 77.0 ppm) or to external acetone (δ_C 31.07 ppm). Optical rotations were measured with a Perkin Elmer 241 polarimeter at 22 °C. Mass spectrometric analysis was performed by positive mode electrospray ionization on a Micromass ZabSpec Hybrid Sector-TOF. For exact measurements, the spectra were obtained by voltage scan over a narrow mass range at 10 000 resolution. MALDI mass spectrometric analysis of protein conjugates was performed on Voyager-elite system from Applied Biosystems.

The allyl aglycon contained in the majority of the mannopyranosides had similar coupling constants throughout and thus are defined and reported only once below.

Ha (dddd, J_{a-c} 10.5 ±0.5 Hz, $^4J_{a-d} \approx {}^4J_{a-e} \approx {}^2J_{a-b}$ 1.5±0.5 Hz), Hb (dddd, J_{b-c} 17.0 ±0.5 Hz, $^4J_{b-d} \approx {}^4J_{b-e} \approx {}^2J_{a-b}$ 1.5 ±0.5 Hz), Hc (dddd, J_{b-c} 17.0 Hz, J_{a-c} 10.5±0.5 Hz, $J_{c-d} \approx J_{c-e}$ 5.5±0.5 Hz), Hd (dddd, J_{d-e} 13.5±0.5 Hz, J_{c-d} 5.5 Hz, $J_{b,d} \approx J_{a,d}$ 1.5±0.5 Hz), He (dddd, $J_{d,e}$ 13.5±0.5 Hz, $J_{c,e}$ 5.5±0.5 Hz, $J_{a,e} \approx J_{b,e}$ 1.5±0.5 Hz)



*Methyl 4-O-benzoyl-2-O-chloroacetyl-3,6-dideoxy-α-D-*arabino-*hexopyranoside* (**4**).

Methyl 4-*O*-benzoyl-3,6-dideoxy-*α-D-arabino*-hexopyranoside (2.1 g, 7.89 mmol) was dissolved in dichloromethane (20 mL) and pyridine was added (1 mL, 12.3 mmol). The solution was cooled to 0 °C and chloroacetic anhydride (2.0 g, 11.8 mmol) was added in one portion. The reaction was allowed to warm to room temperature over 1 h and stirred overnight. The reaction was diluted with dichloromethane, washed with water followed by a brine solution, dried over sodium sulfate and concentrated to a yellow oil. Chromatography in hexane:EtOAc (5:1) gave a colourless oil (2.631 g, 96%); $[\alpha]_D$ 58.3° (*c* 0.52, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) 8.01-7.98 (2 H, m, Ar), 7.58-7.53 (1 H, m, Ar), 7.45-7.40 (2 H, m, Ar), 5.02 (1 H, ddd, J_{1-2}<1, J_{2-3eq} 3.7, J_{2-3ax} 3.2 Hz, H-2), 5.00 (1 H, ddd, J_{3ax-4} 11.4, J_{3eq-4} 4.9, J_{4-5} 9.7 Hz, H-4), 4.60 (1 H, d, H-1), 4.15 (2 H, s, CH_2Cl), 3.99 (1 H, dq, J_{5-6} 6.2 Hz, H-5), 3.42 (3 H, s, OMe), 2.29 (1 H, ddd, J_{gem} 13.7, H-3eq), 2.09 (1 H, ddd, H-3ax), 1.25 (3 H, d, H-6); Anal. Calcd. For $C_{16}H_{19}ClO_6$: C, 55.86; H, 5.69, Found: C, 56.06; H, 5.59.

*1-O-acetyl-4-O-benzoyl-2-O-chloroacetyl-3,6-dideoxy-α-D-*arabino-*hexopyranose* (**5**).

Glycoside **4** (2.5g, 5.7 mmol) was dissolved in acetic anhydride (50 mL) and cooled to 0 °C in an ice bath. Concentrated sulfuric acid (3 drops) was then added and the reaction was allowed to stir for 1 h. The solution was then diluted in dichloromethane and washed with a bicarbonate solution. Drying and concentration gave a colourless oil which was further purified by silica gel chromatography in hexane:EtOAc (4:1). An oil (2.19g, 82%) was obtained which crystallized upon standing. Recrystallization was possible from hexane-diethylether mixtures; m.p. 108-109 °C; $[\alpha]_D$ 65.8° (*c* 0.77, $CHCl_3$); ^{1}H (300 MHz, $CDCl_3$) 8.02-7.99 (2 H, m , Ar), 7.60-7.55 (1 H, m, Ar), 7.47-7.41 (2 H, m, Ar), 6.00 (1 H, d, J_{1-2}<1 Hz, H-1), 5.05 (1 H, ddd, J_{3ax-4} 11.1, J_{3eq-4} 3.6, J_{4-5} 9.7 Hz, H-4), 5.06 (1 H, ddd, $J_{2-3eq} \approx J_{2-3ax}$ 3.6 Hz, H-2), 4.16 (2 H, s, CH_2Cl),

4.05 (1 H, dq, $J_{5\text{-}6}$ 6.1 Hz, H-5), 2.42 (1 H, ddd, J_{gem} 13.9, H-3eq), 2.11 (1 H, ddd, H-3ax), 1.27 (3 H, d, H-6); Anal. Calcd. For $C_{17}H_{19}ClO_7$: C, 55.07; H, 5.17, Found: C, 54.88; H, 5.05.

*Ethyl 4-O-benzoyl-2-O-chloroacetyl-3,6-dideoxy-1-thio-α-D-*arabino*-hexopyranoside* (**6**).

Pyranose **5** (2.1g, 4.5mmol) was dissolved in dry dichloromethane (50 mL) and cooled to –35 °C. Ethane thiol (4.5 mmol, 330 µL) was added followed by boron triflouride diethyl etherate (4.5 mmol, 575 µL). After 1 h another equivalent of each reagent was added, and the reaction was allowed to warm to –10 °C over 3 h. The mixture was then diluted with dichloromethane, washed with a bicarbonate solution, dried over sodium sulfate and concentrated to a yellow oil. Column chromatography in hexane:EtOAc (5:1) gave a colourless oil (1.95g, 92%); $[\alpha]_D$ 123° (*c* 1.1, $CHCl_3$); ^{1}H (300 MHz, $CDCl_3$) 8.03-7.99 (2 H, m , Ar), 7.60-7.54 (1 H, m, Ar), 7.47-7.41 (2 H, m, Ar), 5.21 (1 H, s, H-1), 5.17 (1 H, ddd, $J_{2\text{-}3eq} \approx J_{2\text{-}3ax}$ 3.3 Hz, H-2), 5.05 (1 H, dd, $J_{3ax\text{-}4}$ 10.9, $J_{3eq\text{-}4}$ 4.7, $J_{4\text{-}5}$ 9.6 Hz, H-4), 4.33 (1 H, dq, $J_{5\text{-}6}$ 6.1 Hz, H-5), 4.16 (2 H, s, CH_2Cl), 2.74-2.60 (2H, ABX_3, SCH_2), 2.34 (1 H, ddd, J_{gem} 13.7, H-3eq), 2.07 (1 H, ddd, H-3ax), 1.32 (3 H, t, J 7.4, SCH_2CH_3), 1.26 (3 H, d, H-6); Anal. Calcd. For $C_{17}H_{21}ClO_5S$: C, 54.76; H, 5.68, S, 8.60; Found: C, 54.52; H, 5.77.

*Ethyl 4-O-benzoyl-3,6-dideoxy-1-thio-α-D-*arabino*-hexopyranoside* (**7**).

Thioglycoside **6** (1.9g, 5.1 mmol) was dissolved in absolute ethanol (5 mL) and thiourea was added (1.1 g, 14.5 mmol). The reaction was warmed to 50 °C for 15 h. The ethanol was removed under vacuum and the resulting solid was taken up in dichloromethane. The solution was washed with water, dried over sodium sulfate and concentrated to an oil. Column chromatography in hexane:EtOAc (2:1) gave a colourless syrup (1.38 g, 92 %); $[\alpha]_D$ 162° (*c* 1.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 8.03-8.00 (2 H, m, Ar), 7.58-7.53 (1 H, m, Ar), 7.45-7.40 (2 H, m, Ar), 5.12 (1 H, dd, $J_{3ax\text{-}4}$ 10.2, $J_{3eq\text{-}4}$ 4.6, $J_{4\text{-}5}$ 8.8 Hz, H-4), 5.08 (1 H, s, H-1), 4.33 (1 H, dq, $J_{5\text{-}6}$ 6.1 Hz, H-5), 4.05 (1 H, ddd, $J_{2\text{-}3eq}$ 3.1, $J_{2\text{-}3ax}$ 6.8 Hz, H-2), 2.74-2.59 (2 H, ABX_3, SCH_2), 2.27 (1 H, ddd, J_{gem} 13.4, H-3eq), 2.01 (1 H, ddd, H-3ax), 1.32 (3 H, t, J 7.4, SCH_2CH_3), 1.27 (3 H, d, H-6); Anal. Calcd. For $C_{15}H_{20}O_4S$: C, 60.79; H, 6.80, Found: C, 60.81; H, 6.84.

*Ethylsulfenyl 4-O-benzoyl-3,6-dideoxy-1-thio-α-D-*arabino*-hexopyranoside* (**8**).

Benzoylated thioglycoside **7** (400mg, 1.4 mmol) was dissolved in dichloromethane (7 mL). Silica gel (300 mg) was added followed by acetic anhydride (152 µL, 1.5 mmol) and hydrogen peroxide (30% wt) (186 µL, 1.6 mmol). Small amounts of silica gel were then added

until a smooth heterogeneous mixture was achieved. The reaction was stirred for 5 h and then filtered. The filtrate was washed with a solution of thiosulfate. Drying and concentration gave a white solid that was recrystallized from EtOAc-hexane into fine needles (375 mg, 89%); m.p.115-116 °C; $[\alpha]_D$ 66.8° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 8.00-7.98 (2 H, m, Ar), 7.56-7.53 (1 H, m, Ar), 7.43-7.40 (2 H, m, Ar), 5.13 (1 H, ddd, $J_{3ax-4} \approx J_{4-5}$ 9.4, J_{3eq-4} 4.1 Hz, H-4), 4.62 (1 H, ddd, J_{1-2} 7.0, J_{2-3eq} 4.7, J_{2-3ax} 9.2 Hz, H-2), 4.36 (1 H, d, H-1), 4.10 (1 H, dq, J_{5-6} 6.1 Hz, H-5), 3.00 (1 H, dq, J_{gem} 14.8, J_{vic} 7.62 Hz, SCH_2CH_3), 2.81 (1 H, dq, $SC\underline{H}_2CH_3$), 2.31 (1 H, ddd, J_{gem} 14.4, H-3eq), 2.17 (1 H, ddd, H-3ax), 1.40 (3 H, t, J 7.4, $SCH_2C\underline{H}_3$), 1.33 (3 H, d, H-6); Anal. Calc. For $C_{15}H_{20}O_5S$: C, 57.67; H, 6.45, Found: C, 57.43; H, 6.61.

Ethyl 2-O-*dimethyl-(1*-O-*(2-(trimethylsilyl)ethyl)-4,6*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranos-3-oxyl)-silyl-4*-O-*benzoyl-3,6-dideoxy-1-thio-α-D*-arabino-*hexopyranoside* (**9**).

To a dry round bottom flask were added 2-(trimethylsilyl)-ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (125 mg, 0.251 mmol), **7** (74 mg, 0.251 mmol), imidazole (34 mg, 0.502 mmol) and DMAP (30 mg, 0.251 mmol). The flask was sealed and purged with argon. THF (5 ml) was added and the solution was cooled to -78 °C. Dichlorodimethylsilane (31 μL, 0.255 mmol) was added in one portion and the reaction was allowed to warm to room temperature over 4 h. Stirring was then continued for a further 2 h at which time the reaction was quenched with an aqueous bicarbonate solution. After extracting with dichloromethane, drying over sodium sulfate and concentration a yellow oil remained. This was further purified by column chromatography in hexane:EtOAc (5:1) to give a colourless syrup (82 mg, 44%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.00-7.29 (14 H, m, Ar), 5.54 (1 H, s, $O_2C\underline{H}Ph$), 5.21 (1 H, d, J_{1-2} 8.4 Hz, H-1'), 5.09 (1 H, ddd, J_{3eq-4} 4.6, J_{4-5} 8.1, J_{3ax-4} 10.5, H-4), 5.03 (1 H, s, H-1), 4.80 (1 H, dd, J_{2-3} 10.9, J_{3-4} 3.7, H-3'), 4.61 (1 H, dd, H-2'), 4.32 (1 H, dd, J_{5-6} 1.5, J_{gem}12.2 Hz, H-6a'), 4.24 (1 H, dq, J_{5-6} 6.1 Hz, H-5'), 4.18 (1 H, d, H-4'), 4.10 (1 H, dd, J_{5-6} 1.8, H-6b'), 4.06 (1 H, ddd, H-2), 3.45 (1 H, ddd, J_{vic}5.4, J_{vic}9.8, J_{gem}10.7 Hz, $OC\underline{H}_2CH_2Si$), 3.58 (1 H, br s, H-5), 3.48 (1 H, ddd, J_{vic} 6.7, J_{vic} 10.0, $OC\underline{H}_2CH_2Si$), 2.73-2.57 (2 H, ABX_3, $SC\underline{H}_2CH_3$), 2.08 (1 H, ddd, J_{2-3} 1.4, J_{gem} 13.4 Hz, H-3eq), 1.86 (1H, ddd, J_{2-3} 2.8 Hz, H-3ax), 1.24 (3 H, d, J 7.5, $SCH_2C\underline{H}_3$), 1.23 (3 H, d, H-6), 0.86-0.70 (2 H, ABMX, $CH_2C\underline{H}_2Si$), 0.02 (3 H, $(CH_3)_2Si$), -0.01 (3 H, s, $(CH_3)_2Si$), -0.15 (9 H, s, $Si(CH_3)_3$); ES HRMS *Calcd.* for $C_{35}H_{51}O_{11}N_3SSi_2Na$ 768.2783 found 768.2782.

*2-O-dimethyl-(1-O-(2-(trimethylsilyl)ethyl)-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranos-3-oxyl)-silyl-4-O-benzoyl-3,6-dideoxy-α-D-*arabino-*hexopyranosyl succinimide (10).*

N-iodosuccinimide (15 mg, 0.067mmol) and AgOTf (8 mg, 0.031 mmol) were dried together under vacuum over phosphorus pentaoxide overnight. Silyl acetal (**9**) (50 mg, 0.060 mmol) was dried over night in a separate flask. The flask was flushed with argon and the starting material was dissolved in dry DCM (5 mL). Freshly dried molecular sieves (4A) were then added and the heterogeneous mixture was stirred for 15 min. It was then cooled in an acetone/dry ice bath to –78 °C and the AgOTf and *N*-iodosuccinimide were added. The reaction was slowly brought to room temperature over 3 h. The reaction was then quenched with a bicarbonate solution and filtered through celite. Washing with a thiosulphate solution, drying over sodium sulphate and concentration gave a yelow oil. Further purification over a silica gel column in hexane:EtOAc (4:1) gave a colourless syrup (24 mg, 43%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10-7.28 (14 H, m, Ar), 5.64 (1 H, s, $O_2C\underline{H}Ph$), 5.25 (1 H, d, J_{1-2} 8.5 Hz, H-1'), 5.13 (1H, dd, J_{1-2} 9.0 Hz, H-1), 5.03 (1 H, ddd, J_{2-3} 5, J_{2-3} 11 Hz, H-2), 4.83 (1 H, ddd, J_{3eq-4} 4.6, J_{4-5} ≈J_{3ax-4} 11.0 Hz, H-4), 4.71(1 H, dd, J_{2-3} 10.5, J_{3-4} 3.5 Hz, H-3') 4.47 (1 H, dd, H-2'), 4.27 (1 H, d, H-4'), 4.25-4.14 (3 H, m, H-6', H-5), 4.02 (1 H, ddd, J_{vic} 5.5, J_{gem}≈J_{vic} 9.5 Hz, $OC\underline{H}_2CH_2$), 3.67 (1 H, br s, H-5'), 3.52 (1 H, ddd, J_{vic} 7, J_{vic} 7.5 Hz, $OC\underline{H}_2CH_2$), 2.65 (4 H, s, succinimide), 2.17 (1 H, ddd, J_{gem} 14.3, H-3eq), 1.91 (1H, ddd, H-3ax), 1.27 (3 H, d, J 7.5, H-6), 0.86-0.68 (2 H, ABMX, CH_2CH_2Si), -0.12 (3 H, $(CH_3)_2Si$), -0.16 (12 H, s, $(CH_3)_2Si$, $Si(CH_3)_3$); EMS Calcd. for $C_{35}H_{51}O_9SSi_2$ 909.3 found 909.4.

*Ethylsulfenyl 2-O-dimethyl-(1-O-(2-(trimethylsilyl)ethyl)-2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranos-3-oxyl)-silyl-4-O-benzoyl-3,6-dideoxy-α-D-*arabino-*hexopyranoside* (**11**).

To **9** (80 mg, 0.094 mmol) in dichloromethane (5 mL) was added hydrogen peroxide (30%)(115 µL, 0.12 mmol) followed by acetic anhydride (10 µL, 0.010 mmol). Silica gel was then added to the solution until a homogenous slurry was formed under vigorous stirring. The reaction was left to stir for 3 h then filtered and washed with a solution of thiosulfate. Drying, concentration and flash chromatography of the organics in EtOAc:hexane (1:1), gave a colourless syrup (39 mg, 48%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95-7.26 (14 H, m, Ar), 5.62 (1 H, s, $O_2C\underline{H}Ph$), 5.23 (1 H, d, J_{1-2} 8.6 Hz, H-1'), 5.21 (1 H, ddd, J_{3eq-4} 3.7, J_{4-5} 9.2, J_{3ax-4} 10.9 Hz, H-4), 4.85 (1 H, dd, J_{2-3} 11.0, J_{3-4} 3.7 Hz, H-3'), 4.64 (1 H, dd, H-2'), 4.59 (1 H, br s, H-2), 4.33 (1 h, dd, J_{5-6} 1.4, J_{gem} 12.1 Hz, H-6a'), 4.34 (1H, d, H-4'), 4.27 (1 H, s, H-1), 4.16 (1 H, dd, J_{5-6} 1.8 Hz,

H-6b'), 3.96 (1 H, ddd, J_{vic} 5.1, J_{vic} 8.9, J_{gem} 10.6 Hz, $OC\underline{H}_2CH_2$), 3.78 (1 H, dq, $J_{5\text{-}6}$ 6.2 Hz, H-5), 3.62 (1 H, br s, H-5'), 3.48 (1 H, ddd, J_{vic} 6.8, J_{vic} 4.0 Hz, OCH_2CH_2), 2.83 (1 H, dt, J_{vic} 7.5, J_{gem} 15.2 Hz, SCH_2CH_3), 2.54 (1 H, dt, J_{vic} 7.5 Hz, SCH_2CH_3), 2.26 (1 H, ddd, J_{gem} 13.5 Hz, H-3eq), 1.98 (1 H, ddd, H-3ax), 1.27 (3H, t, J 7.5 Hz, CH_2CH_3), 1.29 (3 H, d, H-6), 0.80 (1 H, ddd, J_{vic} 4.0, J_{vic} 7.3, J_{gem} 10.8 Hz, OCH_2CH_2), 0.72 (1 H, ddd, J_{vic} 5.1, J_{vic} 8.9 Hz, OCH_2CH_2), 0.00 (3 H, s, $Si(CH_3)_2$), -0.05 (3 H, s, $Si(CH_3)_2$), -0.17 (3 H, s, $Si(CH_3)_3$) ES HRMS Calcd. for $C_{43}H_{55}NO_{12}SSi_2Na$ 888.2881 found 888.2885.

2-(Trimethylsilyl)ethyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-β-D-galactoopyranoside **(12).**

3,4,6-Triacetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride (2.5g, 7.2 mmol) was dissolved in dry dichloromethane (20 mL) with freshly flamed molecular sieves (4A)(~1g) and 2-(trimethylsilyl) ethanol (1.4 mL, 0.098 mmol) was added. After stirring for 1 h, the reaction was cooled to –78 °C and silver triflate (2.19 g, 8.6 mmol) was added. The reaction was then allowed to warm to room temperature over 2 h. The reaction was then filtered through celite and washed with a bicarbonate solution. After drying over sodium sulfate and concentration the resulting syrup was chromatographed in hexane:EtOAc (5:1). A colourless syrup was isolated (1.2 g, 40%) along with mixed fractions containing the α anomer (800 mg). ^1H (500 MHz, $CDCl_3$) 5.28 (1 H, d, $J_{3\text{-}4}$ 3.5 Hz, H-4), 4.47 (1 H, dd, $J_{2\text{-}3}$ 10.9 Hz, H-3), 4.33 (1 H, d, $J_{1\text{-}2}$, 8.1 Hz, H-1), 4.15 (1 H, dd, $J_{5\text{-}6}$ 6.4, J_{gem} 11.2 Hz, H-6a), 4.06 (1 H, dd, $J_{5\text{-}6}$ 7.2 Hz, H-6b), 4.00 (1 H, ddd, J_{vic} 6.3, J_{vic} 9.6, J_{gem} 11.0 Hz, $OC\underline{H}_2CH_2$), 3.81 (1 H, dd, H-5) 3.65-3.62 (2 H, m, H-2, $OC\underline{H}_2CH_2$), 2.11 (3 H, s, $COCH_3$), 2.02 (3 H, s, $COCH_3$), 2.00 (3 H, s, $COCH_3$), 1.04 (2 H, ABMX, $CH_2C\underline{H}_2Si$), 0.02 (9 H, s, $Si(C\underline{H}_3)_3$); Anal. Calcd. For $C_{17}H_{29}N_3O_8Si$: C, 47.32; H, 6.77, N, 9.74; Found: C, 47.07; H, 6.89; N, 9.62.

2-(Trimethylsilyl)ethyl 2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside **(13).**

Galactopyranoside **12** (270mg, 0.262 mmol) was dissolved in dry methanol (5 mL) and sodium metal (~5mg) was added. The reaction was stirred for 30 minutes and neutralized with Dowex 50(H^+) resin against pH paper. The resin was removed by filtration through cotton wool and the resulting solution was concentrated to a white solid. This solid was taken up in dry acetonitrile (10 mL) and benzaldehyde dimethylacetal (100μL, 0.657mmol) followed by *p*TSA (5 mg) were added. The reaction was stirred for 1 h and then neutralized with triethylamine. Concentration under vaccum yielded a yellow syrup which was further purified by column chromatography in hexane:EtOAc (3:1) to give a white crystalline solid (220 mg, 89%). m.p.

103-104 °C. $[\alpha]_D$ 18.9° (*c* 1.6, $CHCl_3$) ^1H (500 MHz, $CDCl_3$) 7.47-7.32 (5H, m, Ar), 5.52 (1 H, s, O_2CHPh), 4.30 (1 H, dd, J_{5-6} 1.4, J_{gem} 12.4 Hz, H-6a), 4.25 (1 H, d , J_{1-2} 7.8 Hz, H-1), 4.11 (1 H, d, J_{4-5} 3.7 Hz, H-4), 4.50-4.00 (2 H, m, H-6b Hz, OCH_2CH_2), 3.16-3.55 (2 H, m, H-2, OCH_2CH_2), 3.52 (1 H, *br* s, H-3), 3.39 (1 H, ddd, J_{5-6}<1 Hz, H-5), 1.06-1.02 (2 H, ABMX, CH_2CH_2Si), 0.02 (9 H, s, $Si(CH_3)_3$); Anal. Calcd. For $C_{18}H_{27}N_3O_5Si$: C, 54.94; H, 6.92; N, 10.68, Found: C, 54.90; H, 6.90; N 10.55.

2-(Trimethylsilyl)ethyl 2-azido-4,6-O-benzylidene-2-deoxy-3-O-p-methoxybenzyl-β-D-galactopyranoside **(14).**

Acetal **13** (500mg, 1.28mmol) was dissolved in dry DMF (5 mL), and sodium hydride (95%)(45 mg, 1.86mmol) was added followed by *p*-methoxybenzyl chloride (200 µL, 1.48 mmol). The mixture was stirred for 16 h and diluted with dichloromethane before washing twice with water. The organic layer was then dried over sodium sulfate and concentrated to a yellow syrup. Further purification via column chromatography in hexane:EtOAc (3:1) gave a colourless syrup (620 mg, 94%) which crystallized upon standing. Recrystallization was accomplished in hexane-EtOAc mixtures to yield fine needles; m.p. 74-75 °C. $[\alpha]_D$ 3.1° (*c* 0.8, $CHCl_3$) ^1H (300 MHz, $CDCl_3$) 7.52-7.58 (2 H, m, Ar), 7.35-7.27 (5 H, m, Ar), 6.89-6.82 (2 H, m, Ar), 5.44 (1 H, s, O_2CHPh), 4.64 (2 H, s, OCH_2PhOMe), 4.27 (1 H, dd, J_{5-6} 1.5, J_{gem} 12.4 Hz, H-6a), 4.22 (1 H, d, J_{1-2} 8.1 Hz, H-1), 4.06-3.96 (3 H, m, H-4, H-6b, OCH_2CH_2), 3.80 (1 H, dd, J_{2-3} 10.4 Hz, H-2), 3.78 (3 H, s, $PhOCH_3$), 3.55 (1 H, ddd, J_{vic} 7.6, J_{vic} 9.5, J_{gem} 14.5 Hz, OCH_2CH_2), 3.32 (1 H, dd, J_{3-4} 3.5 Hz, H-3) 3.25 (1 H, ddd, J_{4-5}<1 H, J_{5-6}<1 Hz, H-5), 1.10, 0.94 (2 H, ABMX, CH_2CH_2Si), 0.02 (9 H, s, $Si(CH_3)_3$; Anal. Calcd. For $C_{26}H_{35}N_3O_6Si$: C, 60.79; H, 6.87; N, 8.18; Found: C, 60.79; H, 6.86; N, 8.20.

Ethyl 2-O-dimethyl-(1-O-(2-(trimethylsilyl)ethyl)-2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranos-3-oxyl)-silyl-4-O-benzoyl-3,6-dideoxy-1-thio-α-D-arabino-hexopyranoside **(15).**

To a dry round bottom flask were added **13** (70 mg, 0.176 mmol), **7** (52 mg, 0.176 mmol), imidazole (24 mg, 0.352 mmol) and DMAP (21mg, 0.176 mmol). The flask was sealed and purged with argon. THF (5 ml) was added and the solution was cooled to -78 °C. Dichlorodimethyl silane (22 µL, 0.176 mmol) was added in one portion and the reaction was allowed to warm to room temperature over 4 h. Stirring was then continued for a further 1 h at which time the reaction was quenched with an aqueous bicarbonate solution. The mixture was

extracted with dichloromethane, dried over sodium sulfate and concentrated a yellow oil. The oil was further purified by column chromatography in hexane-EtOAc (5:1) to give a colourless syrup (27 mg, 22%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97-7.96 (2 H, m, Ar), 7.54-7.24 (8 H, m, Ar), 5.49 (1 H, s, $O_2C\underline{H}Ph$), 5.15 (1 H, ddd, J_{3eq-4} 4.6, $J_{4-5} \approx J_{3ax-4}$ 11.0 Hz, H-4), 5.11 (1 H, s, H-1), 4.31-4.28 (2H, m, H-5, H-6a'), 4.27 (1 H, d, J_{1-2} 8.1 Hz, H-1'), 4.23 (1 H, *br* s, H-2), 4.18 (1 H, d, J_{3-4} 3.7 Hz, H-4'), 4.11 (1 H, dd, J_{5-6} 1.3, J_{gem}12.1 Hz, H-6b'), 4.04 (1 H, ddd, J_{vic} 6.4, J_{vic} 9.7, J_{gem}10.8 Hz, $OC\underline{H}_2CH_2Si$), 3.83 (1 H, dd, J_{2-3} 10.9, J_{3-4} 3.7 Hz, H-3'), 3.72 (1 H, dd, H-2'), 3.58 (1 H, ddd, J_{vic} 6.7, J_{vic} 10.0 Hz, $OC\underline{H}_2CH_2Si$), 3.42 (1 H, br s, H-5'), 2.67-2.53 (1 H, ABX_3, $SC\underline{H}_2CH_3$), 2.20 (1 H, ddd, J_{2-3} 1.4, J_{gem} 13.0 Hz, H-3eq), 1.90 (1H, ddd, J_{2-3} 2.8 Hz, H-3ax), 1.28 (3 H, d, J 7.5 Hz, $SCH_2C\underline{H}_3$), 1.24 (3 H, d, H-6), 1.08-0.98 (2 H, ABMX, $CH_2C\underline{H}_2Si$), 0.24 (3 H, $(CH_3)_2Si$), 0.21 (3 H, s, $(CH_3)_2Si$), 0.01 (9 H, s, $Si(CH_3)_3$); ES HRMS Calcd. for $C_{35}H_{51}N_3O_9SSi_2Na$ 768.2790 found 768.2782

*2-(Trimethylsilyl)ethyl (4-O-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranosyl)(1→3) 2-azido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside.*(**16**)

Glycosyl sulphoxide **8** (88 mg 0.28 mmol) and alcohol **14** (64 mg 0.28mmol) were added to a round bottom flask followed by freshly dried molecular sieves (4A). The flask was flushed with argon, dichloromethane (2 mL) was added and the reaction was allowed to stir for 1 h before proceeding. The flask was then cooled to –10 °C and DDQ (64 mg, 0.28 mmol) was added. The reaction was allowed to reach room temperature over 4 h,was then quenched with the addition of 1,4-cyclohexadiene. The sieves were removed by filtration through celite. The flitrate was washed with an aqueous bicarbonate solution. After drying over sodium sulfate and concentration a colourless oil was obtained. This was dried under high vacuum for 2 h. 2,6-Di-*tert*-butyl-4-methylpyridine (126 mg, 0.62 mmol) and 4A molecular seives were added and then the mixture was taken up in dry dichloromethane (2 mL). The solution was cooled to –78 °C and triflouromethanesulfonic anhydride (47 μL, 0.28 mmol) was added. The reaction was stirred for 30 min and then quenched with the addition of an aqueous bicarbonate solution. The reaction was filtered through celite, washed with water and concentrated. The resulting yellow oil was further purified by chromatography in hexane:EtOAc (4:1). A colourless syrup, compound **16** (52 mg 36%) was recovered from the column. ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.05-7.99 (2 H, m, Ar), 7.65-7.35 (8 H, m, Ar), 5.60 (1 H, s, $O_2C\underline{H}Ph$), 5.02 (1 H, ddd, J_{3eq-4} 4.5, J_{4-5} 9.0, J_{3ax-4} 10.5 Hz, H-4'), 4.89 (1 H, s, H-1'), 4.35 (1 H, d, J_{1-2} 7.7 Hz, H-1), 4.28-4.24 (2H, m, H-6a, H-4), 4.11-4.02 (3 H, m, H-2', H-6b, $OC\underline{H}_2CH_2$), 3.83-3.62 (3 H, m, H-2, H-3, H-5', OCH_2CH_2), 2.52 (1 H, *br* s, H-

5), 2.44 (1 H, ddd, J gem 13.6, J_{2-3}≈J_{3-4} 4.4 Hz, H-3eq'), 1.73 (1 H, ddd, J_{2-3} 3.2, J_{3-4} 10.8 Hz, H-3ax'), 1.30 (3 H, d, J 6.2 Hz, H-6'), 1.07 (2 H, ABMX, CH_2CH_2Si) ^{13}C NMR (125 MHz, CD_2Cl_2) δ 102.4 ($^1J_{C-H}$ 160.9 Hz, C-1'), 102.2 ($^1J_{C-H}$ 158.0 Hz, C-1); ES HRMS Calcd. for $C_{31}H_{41}N_3O_9Si$ 627.2612 found 627.2614.

*Methyl 4-O-benzoyl-2-O-acetyl-3,6-dideoxy-α-D-*arabino*-hexopyranoside* (**17**).

Methyl 4-O-benzoyl-3,6-dideoxy-*α-D-arabino*-hexopyranoside (1.2 g, 3.90mmol) was dissolved in dichloromethane (25 mL) and pyridine was added (4 mL). The solution was cooled to 0 °C and acetic anhydride (4.0 mL) was added in one portion. The reaction was allowed to warm to room temperature and stirred for 4 h. The reaction was then concentrated to a yellow oil. Chromatography in hexane:EtOAc (2:1) gave a colourless oil (1.35 g, 97%), $[\alpha]_D$ 81.3° (*c* 0.67, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 8.02-7.97 (2 H, m, Ar), 7.59-7.53 (1 H, m, Ar), 7.45-7.41 (2 H, m, Ar), 5.04 (1 H, ddd, J_{3ax-4}≈J_{4-5} 11.1, J_{3eq-4} 4.8 Hz, H-4), 4.95 (1 H, ddd, J_{1-2}<1, J_{2-3eq} 1.9, J_{2-3ax} 3.2 Hz, H-2), 4.57 (1 H, d , H-1), 3.97 (1 H, dq, J_{5-6} 6.0 Hz, H-5), 3.42 (3 H, s, OMe), 2.25 (1 H, ddd, J_{gem} 13.5 Hz, H-3eq), 2.13 (3 H, s, CH_3CO), 2.05 (1 H, ddd, H-3ax), 1.26 (3 H, d, H-6); Anal. Calcd. For $C_{16}H_{20}O_6$: C, 62.33; H, 6.54, Found: C, 62.27; H, 5.57

*1-O-acetyl-4-O-benzoyl-2-O-acetyl-3,6-dideoxy-α-D-*arabino*-hexopyranose* (**18**).

Methyl glycoside **17** (1.2 g, 3.9 mmol) was dissolved in acetic anhydride (50 ml) and cooled to 0 °C in an ice bath. Concentrated sulfuric acid (200 µl) was then added and the reaction was stirred for 1 h. The solution was then diluted in dichloromethane and washed with a bicarbonate solution. Drying over sodium sulfate and concentration gave a colourless oil which was further purified by silica gel chromatography in hexane:EtOAc (2:1). An oil (1.17 g, 84 %) was obtained which crystallized upon standing. Recrystallization was possible with hexane-diethylether mixtures; m.p. 100-102 °C; $[\alpha]_D$ 71.7° (*c* 1.1 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 8.03-7.99 (2 H, m, Ar), 7.60-7.54 (1 H, m, Ar), 7.47-7.42 (2 H, m, Ar), 5.96 (1 H, d, J_{1-2}<1 Hz, H-1), 5.08 (1 H, ddd, J_{3ax-4}≈J_{4-5} 10.9, J_{3eq-4} 4.8 Hz, H-4), 4.97 (1 H, ddd, J_{2-3eq} 3.1, J_{2-3ax} 4.6 Hz, H-2), 4.04 (1 H, dq, J_{5-6} 6.1 Hz, H-5), 2.37 (1 H, ddd, J_{gem} 13.6 Hz, H-3eq), 2.15 (6 H, s, CH_3CO), 2.07 (1 H, ddd, H-3ax), 1.27 (3 H, d, H-6); Anal. Calcd. For $C_{17}H_{20}O_7$: C, 60.71; H, 5.99, Found: C, 60.72; H, 5.91

*4-O-benzoyl-3,6-dideoxy-1,2-O-(exo-methoxyethylidene)-β-D-*arabino*-hexopyranose* (**19**).

Pyranose **18** (1.0 g, 3.0 mmol) was added to a dry round bottom flask and cooled to 0 °C in an ice bath. Hydrogen bromide in acetic acid (33%) (5 mL) was added and the reaction was stirred for 1 h at 0 °C. The reaction was then diluted with dry toluene (50 mL) and concentrated. The resulting orange oil was dissolved in dichloromethane and washed with a saturated bicarbonate solution followed by a thiosulfate solution (5%). After drying over sodium sulfate the solution was concentrated to a colourless oil and dried under vacuum. The oil was taken up in dry dichloromethane (5 ml) and lutidine was added (1 ml) followed by dry methanol (300 µL). The reaction was stirred overnight, diluted with dichloromethane, washed with water, dried over sodium sulfate and concentrated to a syrup. Chromatography in hexane:EtOAc (3:1) containing 2% triethylamine gave a colourless syrup (662 mg, 72%) which could be crystallized from diethyl ether-hexane mixtures; m.p. 87-88 °C; $[\alpha]_D$ 34° (*c* 0.51 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 8.03-7.99 (2 H, m, Ar), 7.62-7.56 (1 H, m, Ar), 7.47-7.43 (2 H, m, Ar), 5.37 (1 H, d, $J_{1\text{-}2}$ 2.8 Hz, H-1), 4.91 (1 H, ddd, $J_{3ax\text{-}4}$ 9.9, $J_{4\text{-}5}$ 8.3, $J_{3eq\text{-}4}$ 4.7 Hz, H-4), 4.40 (1 H, ddd, $J_{2\text{-}3eq} \approx J_{2\text{-}3ax}$ 2.8 Hz, H-2), 3.68 (1 H, dq, $J_{5\text{-}6}$ 6.2 Hz, H-5), 3.26 (3 H, s, OCH_3), 2.58 (1 H, ddd, J_{gem} 14.4 Hz, H-3eq), 1.96 (1 H, ddd, H-3ax), 1.66 (3 H, s, $C\underline{H}_3CO_3$), 1.27 (3 H, d, H-6); Anal. Calcd. For $C_{16}H_{20}O_6$: C, 62.33; H, 6.54, Found: C, 62.23; H, 6.37.

*4-O-benzyl-3,6-dideoxy-1,2-O-(exo-methoxyethylidene)-β-D-*arabino-*hexopyranoside* (**20**).

Orthoester **19** (514 mg, 1.67 mmol) was dissolved in dry methanol (15 mL) and sodium metal (~20 mg) was added. The reaction was stirred under argon for 2 h and concentrated to dryness. The resulting oil was taken up in DMF (10 ml) and sodium hydride (65%)(92 mg, 2.6 mmol) was added followed by benzyl bromide (300 µL, 2.5mmol). The reaction was stirred for 1 h then quenched with the addition of methanol. The resulting mixture was diluted with EtOAc and washed with water. After drying over sodium sulfate and concentration the resulting syrup was purified by chromatography in hexane:EtOAc (3:1) containing 2% triethylamine to give a colourless oil (426 mg, 87%) $[\alpha]_D$ 52.1° (*c* 1.0 $CHCl_3$); ^{1}H (300 MHz, $CDCl_3$) 7.37-7.26 (5 H, m, Ar), 5.31 (1 H, d, $J_{1\text{-}2}$ 2.8 Hz, H-1), 4.60 (1 H, d, J 11.5 Hz, $OC\underline{H}_2Ph$), 4.48 (1 H, d, $OC\underline{H}_2Ph$), 4.35 (1 H, ddd, $J_{2\text{-}3eq}$ 2.9, $J_{2\text{-}3ax}$ 5.4 Hz, H-2), 3.45 (1 H, dq, $J_{4\text{-}5}$ 7.8, $J_{5\text{-}6}$ 6.2 Hz, H-5), 3.23 (3 H, s, OCH_3), 3.35(1 H, ddd, $J_{3ax\text{-}4}$ 9.4, $J_{3eq\text{-}4}$ 4.3 Hz, H-4), 2.48 (1 H, ddd, J_{gem} 14.5 Hz, H-3eq), 1.79 (1 H, ddd, H-3ax), 1.59(3 H, s, $C\underline{H}_3CO_3$), 1.28 (3 H, d, H-6); Anal. Calcd. For $C_{16}H_{22}O_5$: C, 65.29; H, 7.53, Found: C, 65.33; H, 7.72.

*Ethyl 4-O-benzyl-2-O-acetyl-3,6-dideoxy-1-thio-α-D-*arabino-*hexopyranoside* (**21**).

Benzylated orthoester **20** (400mg, 1.36mmol) was dissolved in acetonitrile (5 mL) and ethanethiol (1 mL) was added. The reaction was cooled to 0 °C and TMSOTf (10 μL) was added. The reaction was stirred for 1 h and then allowed to warm to room temperature. The reaction was quenched with triethylamine and concentrated under vacuum. Column chromatography in hexane:EtOAc (3:1) gave a colourless oil (327mg, 74%); $[\alpha]_D$ 180° (*c* 1.0 $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.25 (5 H, m, Ar), 5.10 (1 H, s, H-1), 5.03 (1 H, ddd, $J_{1\text{-}2}$ 1.6, $J_{2\text{-}3eq} \approx J_{2\text{-}3ax}$ 3.1 Hz, H-2), 4.60 (1 H, d, J 11.4, $OC\underline{H}_2Ph$), 4.46 (1 H, d, $OC\underline{H}_2Ph$), 4.08 (1 H, dq, $J_{4\text{-}5}$ 9.2, $J_{5\text{-}6}$ 6.2 Hz, H-5), 3.38 (1 H, ddd, $J_{3ax\text{-}4}$ 11.2, $J_{3eq\text{-}4}$ 4.4 Hz, H-4), 2.65-2.55 (2 H, ABX_3, $SC\underline{H}_2CH_3$), 2.21 (1 H, ddd, J_{gem} 13.7 Hz, H-3eq), 2.07 (3 H, s, $COCH_3$), 1.85 (1 H, ddd, H-3ax), 1.29 (3 H, t, J 7.3, $SCH_2C\underline{H}_3$), 1.26 (3 H, d, H-6); Anal. Calcd. For $C_{17}H_{24}O_4S$: C, 62.93; H, 7.46, Found: C, 63.12; H, 7.52.

*Ethyl 4-O-benzyl-3,6-dideoxy-1-thio-α-D-*arabino-*hexopyranoside* (**22**).

Thioglycoside **21** (300mg, 0.93mmol) was dissolved in dry methanol (15 mL) and sodium metal (~20 mg) was added. The reaction was stirred for 1 h and then quenched with the addition of Dowex 50 (H^+) resin until neutral on pH indicator paper. After filtration and concentration a colourless oil (256 mg, 98%) remained. $[\alpha]_D$ 230° (*c* 0.8 $CHCl_3$); ^{1}H (600 MHz, $CDCl_3$) 7.34-7.24 (5 H, m, Ar), 5.01 (1 H, s, H-1), 4.60 (1 H, d, J 11.6, $OC\underline{H}_2Ph$), 4.46 (1 H, d, $OC\underline{H}_2Ph$), 4.11 (1 H, dq, $J_{4\text{-}5}$ 8.8, $J_{5\text{-}6}$ 6.3 Hz, H-5), 4.00 (1 H, dd, $J_{2\text{-}3eq}$ 3.7, $J_{2\text{-}3ax}$ 5.5 Hz, H-2), 3.44(1 H, ddd, $J_{3ax\text{-}4}$ 10.5, $J_{3eq\text{-}4}$ 4.5 Hz, H-4), 2.68-2.53 (2 H, ABX_3, $SC\underline{H}_2CH_3$), 2.29 (1 H, ddd, J_{gem} 13.4 Hz, H-3eq), 1.80 (1 H, ddd, H-3ax), 1.27 (3 H, t, J 7.3 Hz, $SCH_2C\underline{H}_3$), 1.26 (3 H, d, H-6); Anal. Calcd. For $C_{15}H_{22}O_3S$: C, 63.80; H, 7.85, Found: C, 63.81; H, 7.94.

*Ethyl 4-O-benzyl-3,6-dideoxy-2-O-p-methoxybenzyl-1-thio-α-D-*arabino-*hexopyranoside* (**23**).

The thioglycoside **22** (204mg, 0.72 mmol) was dissolved in DMF (5 mL) and sodium hydride (65%)(32mg 0.90 mmol) was added followed by *p*-methoxybenzyl chloride (125μL, 0.79mmol). The reaction was stirred for 3 h, quenched with methanol and diluted with EtOAc. After washing with water, drying over sodium sulfate and concentration, a yellow oil was obtained. Column chromatography in hexane EtOAc (5:1) gave a colourless syrup (247 mg, 86%); $[\alpha]_D$ 130° (*c* 0.70 $CHCl_3$); ^{1}H (300 MHz, $CDCl_3$) 7.34-7.22 (7 H, m, Ar), 6.89-6.85 (2 H, m, Ar), 5.21 (1 H, s, H-1), 4.57 (1 H, d, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.53 (1 H, d, J 12.2 Hz, $OC\underline{H}_2Ph$), 4.46 (1 H, d, J 11.5 Hz, $OC\underline{H}_2Ph$), 4.41 (1 H, d, J 11.8 Hz, $OC\underline{H}_2Ph$), 4.07 (1 H, dq, $J_{4\text{-}5}$ 10.9, $J_{5\text{-}6}$ 7.1 Hz, H-5), 3.80 (3 H, s, OCH_3), 3.69 (1 H, dd, $J_{2\text{-}3eq} \approx J_{2\text{-}3ax}$ 3.3 Hz, H-2), 3.48 (1 H, ddd, $J_{3ax\text{-}4}$

4.3, J_{3eq-4} 9.5 Hz, H-4), 2.69-2.49 (2 H, ABX_3, SCH_2CH_3), 2.22 (1 H, ddd, J_{gem} 13.4 Hz, H-3eq), 1.71 (1 H, ddd, H-3ax), 1.29 (3 H, t, J 6.1 Hz, SCH_2CH_3), 1.26 (3 H, d, H-6); Anal. Calcd. For $C_{23}H_{30}O_4S$: C, 68.62; H, 7.51, Found: C, 68.66; H, 7.52.

Ethylsulfenyl 4-O-benzyl-3,6-dideoxy-2-O-p-methoxybenzyl-α-D-arabino-hexopyranoside (**24**).

Thioglycoside **22** (212mg, 0.53 mmol) was dissolved in dichloromethane (4 mL), silica gel (200 mg) was added followed by acetic anhydride (58 µL, 0.57 mmol) and hydrogen peroxide (30% wt) (70 µL, 0.61 mmol). Small amounts of silica gel were then added until a smooth heterogeneous mixture was achieved. The reaction was stirred for 5 h then filtered and washed with a solution of thiosulfate. Drying and concentration gave a white solid that was recrystallized from EtOAc-hexane into fine needles (193 mg, 87%); m.p.. 112-115 °C; $[\alpha]_D$ 115° (*c* 0.80 $CHCl_3$); ^1H (300 MHz, $CDCl_3$) 7.34-7.19 (7 H, m, Ar), 6.87-6.85 (2 H, m, Ar), , 4.56 (1 H, d, J 11.4 Hz, OCH_2Ph), 4.52 (1 H, d, J 11.5 Hz, OCH_2Ph), 4.47 (1 H, s, H-1), 4.45 (1 H, d, J 12.5 Hz, OCH_2Ph), 4.43 (1 H, d, J 11.5 Hz, OCH_2Ph), 4.26 (1 H, ddd, J_{1-2}=1.1, $J_{2-3eq} \approx J_{2-3ax}$ 3.3 Hz, H-2), 3.80 (3H, s, OCH_3), 3.55 (1 H, dq, J_{4-5} 10.9, J_{5-6} 7.1 Hz, H-5), 3.50 (1 H, ddd, J_{3ax-4} 4.0, J_{3eq-4} 9.2 Hz, H-4), 2.95 (1 H, dq, J_{vic} 7.7, J_{gem} 13.5 Hz, SCH_2CH_3), 2.68 (1 H, dq, J_{vic} 7.7 Hz, SCH_2CH_3), 2.40 (1 H, ddd, J_{gem} 13.9 Hz, H-3eq), 1.83 (1 H, ddd, H-3ax), 1.36 (3 H, t, SCH_2CH_3), 1.25 (3 H, d, H-6); Anal. Calcd. For $C_{23}H_{30}O_5S$: C, 66.00; H, 7.22, Found: C, 65.72; H, 7.43.

Methyl 2,4,6-O-triacetyl-3-deoxy-α-D-arabino-hexopyranoside (**25**).

Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (1.0g, 3.8 mmol) was dissolved in 80% acetic acid and warmed to 60 °C for 4 h. The resulting solution was cooled and concentrated under vacuum. The colourless oil was then taken up in pyridine (25 mL), acetic anhydride (5 mL) was added and the reaction was stirred for 16 h. The volatiles were removed from the yellow solution and the resulting oil was azeotroped from toluene (30 mL) twice. The remaining syrup was chromatographed in EtOAc:hexane (2:1) to give a colourless oil (1.04 g, 92%); $[\alpha]_D$ 63.2° (*c* 0.53, $CHCl_3$) ^1H (300 MHz, $CDCl_3$) 4.98 (1 H, ddd, $J_{3ax-4} \approx J_{4-5}$ 10.6, J_{3eq-4} 5.0 Hz, H-4), 4.88 (1 H, dd, J_{2-3eq} 3.2, J_{2-3ax} 4.5 Hz, H-2), 4.59 (1 H, s, H-1), 4.23 (1 H, dd, J_{5-6} 5.8, J_{gem} 12.0 Hz, H-6a), 4.13 (1 H, dd, J_{5-6} 2.6 Hz, H-6b), 3.91 (1 H, ddd, J_{5-6} 6.2 Hz, H-5), 3.38 (3 H, s, OMe), 2.17 (1 H, ddd, J_{gem} 13.7 Hz, H-3eq), 2.09 (3 H, s, $COCH_3$), 2.08 (3 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$), 1.95 (1 H, ddd, H-3ax), Anal. Calcd. For $C_{13}H_{20}O_8$: C, 51.31; H, 6.62, Found: C, 51.00; H, 6.63.

1-O-Acetyl-2,4,6-O-triacetyl-3-deoxy-α-D-arabino-hexopyranose **(26).**

25 (946mg, 3.1 mmol) was dissolved in acetic anhydride (20 ml) and cooled to 0 °C in an ice bath. Concentrated sulfuric acid (3 drops) was then added and the reaction was allowed to stir for 1 h. This solution was then diluted in dichloromethane and washed with a bicarbonate solution. Drying and concentration gave a colourless oil which was further purified by silica gel chromatography in hexane:EtOAc (2:1) giving an oil (866 mg, 84 %); $[\alpha]_D$ 55° (*c* 0.98, $CHCl_3$); ^1H (300 MHz, $CDCl_3$) 5.93 (1 H, s, H-1), 5.00 (1 H, ddd, $J_{3ax-4} \approx J_{4-5}$ 11.0, J_{3eq-4} 4.9 Hz, H-4), 4.87 (1 H, dd, J_{2-3eq} 1.5, J_{2-3ax} 3.2 Hz, H-2), 4.20 (1 H, dd, J_{5-6} 5.3, J_{gem} 12.2 Hz, H-6a), 4.08 (1 H, dd, J_{5-6} 2.4 Hz, H-6b), 3.95 (1 H, ddd, J_{5-6} 6.2 Hz, H-5), 2.25 (1 H, ddd, J_{gem} 13.7 Hz, H-3eq), 2.09 (6 H, s, $2(COCH_3)$), 2.04 (3 H, s, $COCH_3$), 2.01 (3 H, s, $COCH_3$), 1.95 (1 H, ddd, H-3ax), Anal. Calcd. For $C_{14}H_{20}O_9$: C, 50.60; H, 6.03, Found: C, 50.40; H, 6.19.

Ethyl 2,4,6-O-triacetyl-3-deoxy-1-thio-α-D-arabino-hexopyranoside **(27).**

Thioglycoside **26** (832mg, 2.51 mmol) was dissolved in dry dichloromethane (30 mL) and cooled to 0 °C. Ethanethiol (5.0 mmol, 371 μL) was added followed by $BF_3{\cdot}Et_2O$ (5.10 mmol, 642 μL) and the reaction was allowed to stir for 3 h warming to room temperature. The mixture was then diluted with dichloromethane, washed with a bicarbonate solution, dried over sodium sulfate and concentrated to a yellow oil. Column chromatography in hexane:EtOAc (3:1) gave a colourless oil (837mg, 87%); $[\alpha]_D$ 121° (*c* 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.25 (1 H, s, H-1), 5.00-4.91 (2 H, m , H-2, H-4), 4.30-4.18 (2 H, m, H-5, H-6a), 4.06 (1 H, dd, J_{gem} 11.7, J_{5-6} 2.1 Hz, H-6b), 2.66-2.47 (2H, ABX_3, SCH_2CH_3), 2.15 (1 H, ddd, J_{2-3} 1.4, J_{3-4} 3.1, J_{gem} 13.7 Hz, H-3eq), 2.05 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 1.97 (3 H, s, $COCH_3$), 1.85 (1 H, ddd, J_{2-3} 3.2, J_{3-4} 11.2 Hz, H-3ax), 1.23 (3 H, t, J 7.4 Hz, SCH_2CH_3); Anal. Calcd. For $C_{14}H_{22}O_7S$: C, 50.20; H, 6.72; Found: C, 50.29; H, 6.63.

Ethyl 4,6-O-benzylidene-3-deoxy-1-thio-α-D-arabino-hexopyranoside **(28).**

Acetylated glycoside **27** (798mg, 2.39 mmol) was dissolved dry methanol (10 mL) and sodium metal was added (~25 mg). The reaction was allowed to stir for 3 h and then concentrated to dryness. The residue was taken up in acetonitrile (10 mL) and benzaldehyde dimethylacetal (6.6mmol, 1 ml) was added followed by *p*TSA until the reaction was acidic as indicated by litmus paper. The reaction was stirred for 2 h and neutralized with triethylamine. The solution was filtered through celite and concentrated to a yellow oil. Chromatography in hexane:EtOAc (3:1) gave a colourless syrup (580 mg, 82%) which crystallized upon standing. Recrystallization from

hexane-EtOAc mixtures gave robust needles; m.p. 127-128 °C; $[\alpha]_D$ 247° (*c* 1.1, $CHCl_3$); ^1H (300 MHz, $CDCl_3$) δ 7.48-7.31 (2 H, m, Ar), 7.38-7.31 (3 H, m Ar), 5.56 (1 H, s, O_2CHPh), 5.15 (1 H, s, H-1), 4.28-4.11 (3 H, m, H-2, H-4, H-6a), 4.01 (1 H, ddd, J_{5-6} 4.8, J_{5-6} 11.8, J_{4-5} 9.2 Hz, H-5), 3.80 (1 H, dd, J_{gem} 10.2 Hz, H-6b), 2.72-2.52 (2H, ABX_3, $SC\underline{H}_2CH_3$), 2.13 (1 H, ddd, J_{2-3} 1.4, J_{3-4} 3.1, J_{gem} 13.6 Hz, H-3eq), 2.03 (1 H, ddd, J_{2-3} 3.0, J_{3-4} 10.9 Hz, H-3ax), 1.28 (3 H, t, J 7.4, $SCH_2C\underline{H}_3$); Anal. Calcd. For $C_{15}H_{20}O_4S$: C, 60.79; H, 6.80; Found: C, 60.58; H, 6.87.

*Ethyl 4,6-O-benzylidene-3-deoxy-2-p-methoxybenzyl-1-thio-α-D-*arabino-*hexopyranoside* (**29**).

Acetal **28** (523mg, 1.77mmol) was dissolved in DMF (5 ml), sodium hydride (65%)(75mg, 2.1 mmol) followed by *p*-methoxybenzyl chloride (2.1 mmol, 335 μL) were added and the reaction was stirred for 3 h. The mixture was then quenched with the addition of methanol, diluted with EtOAc and washed with water. The organic layer was then dried over sodium sulfate and concentrated to a yellow oil. Chromatography in hexane:EtOAc (4:1) gave a colourless syrup (654mg, 89%) which crystallized upon standing. Recrystallization was possible from EtOAc-hexane mixtures to give fine needles; m.p. 108-109 °C; $[\alpha]_D$ 133° (*c* 1.2, $CHCl_3$); ^1H (600 MHz, $CDCl_3$) δ 7.48-7.31 (2 H, m, Ar), 7.38-7.31 (5 H, m Ar), 6.89-6.87 (2 H, m, Ar), 5.56 (1 H, s, O_2CHPh), 5.28 (1 H, s, H-1), 4.55 (1 H, d, J 11.5 Hz, OCH_2Ph), 4.50 (1 H, d, OCH_2Ph), 4.19-4.16 (2 H, m , H-5, H-6a), 4.03 (1 H, ddd, J_{3eq-4} 4.2, J_{4-5} 9.3, J_{3ax-4} 11.9 Hz, H-4), 3.83-3.78 (5 H, m, H-2, H-6b, OCH_3), 2.72-2.52 (2 H, ABX_3, $SC\underline{H}_2CH_3$), 2.21 (1 H, ddd, J_{2-3} 1.5, J_{gem} 13.0 Hz, H-3eq), 2.03 (1 H, ddd, J_{2-3} 2.9 Hz, H-3ax), 1.27 (3 H, t, J 7.4 Hz, $SCH_2C\underline{H}_3$); Anal. Calcd. For $C_{15}H_{20}O_4S$: C, 66.32; H, 6.78; Found: C, 66.05; H, 6.87.

*Ethylsulfenyl 4,6-O-benzidiene-3-deoxy-1-thio-α-D-*arabino-*hexopyranoside* (**30**).

Acetal **28** (326mg, 1.10mmol) was dissolved in dichloromethane (5 mL) and silica gel (235 mg) was added followed by acetic anhydride (119 μL, 1.2 mmol) and hydrogen peroxide (30% wt) (146 μL, 1.2 mmol). Small amounts of silica gel were then added until a smooth heterogeneous mixture was achieved. The reaction was stirred for 5 h then filtered and washed with an aqueous solution of thiosulfate. Drying and concentration gave a white solid that was recrystallized from EtOAc-hexane into fine needles (298 mg, 87%); m.p.119-122 °C; $[\alpha]_D$ 98° (*c* 1.0, $CHCl_3$); ^1H (600 MHz, $CDCl_3$) δ 7.45-7.34 (5 H, m, Ar), 5.57 (1 H, s, O_2CHPh), 4.73 (1 H, dd, $J_{2-3ax} \approx J_{2-3eq}$ 3.0 Hz, H-2) 4.48 (1 H, s, H-1), 4.18 (1 H, dd, J_{5-6} 4.9, J_{gem} 10.4 Hz, H-6a), 4.13 (1 H, ddd, J_{3eq-4} 4.2, J_{3ax-4} 12.1, J_{4-5} 9.2 Hz, H-4), 3.73 (1 H, dd, J_{5-6} 10.1 Hz, H-6b), 3.63 (1 H, dd, H-5), 3.01 (1 H, dq, J_{gem} 13.6, J_{vic} 7.5 Hz, $SC\underline{H}_2CH_3$), 2.72 (1 H, dq, J_{vic} 7.5 Hz, $SC\underline{H}_2CH_3$), 2.34 (1

H, ddd, J_{gem} 13.3 Hz, H-3eq), 2.17 (1 H, ddd, H-3ax), 1.40 (1 H, t, $SCH_2C\underline{H}_3$); Anal. Calcd. For $C_{15}H_{20}O_5S$: C, 57.67; H, 6.45; Found: C, 57.54; H, 6.58.

Methyl 3,4,6-tri-deoxy-β-D-glycero-hex-3-en-2-ulo-pyranoside (**31**).

Methyl 4-O-benzoyl-3,6-dideoxy-β-D-*arabino*-hexopyranoside (190 mg, 0.71 mmol) was dissolved in DMSO (6 mL) and acetic anhydride was added (3 mL). The reaction was stirred overnight and extracted with dichloromethane. After drying an concentration column chromatography in hexane:EtOAc (3:1) gave a colourless oil (50 mg, 49%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95 (1 H, dd, J_{3-4} 13.2, J_{4-5} 2.1 Hz, H-4), 6.15 (1 H, d, H-3), 4.78 (1 H, s, H-1), 4.60 (1 H, dq, J_{5-6} 6.2 Hz, H-5), 3.54 (3 H, s, OMe), 1.52 (3 H, d, H-6); ESMS Calcd. For $C_7H_{10}O_3Na$:165.06 found 165.1.

Methyl 4-O-benzyl-3,6-dideoxy-β-D-erythro-2-ulo-pyranoside (**32**).

Methyl 4-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranoside (70 mg, 0.28 mmol) was dissolved in dry DMSO (6 mL) and acetic anhydride (2 mL) was added at rt. The reaction was allowed to stir over night and was then extracted with dichloromethane. Concentration followed by column chromatography in hexane:EtOAc (4:1) gave a colourless syrup (63 mg, 90%); ^{1}H NMR (300 MHz, $CHCl_3$): δ 7.34-7.24 (5 H, m, Ar), 4.66 (1 H, s, H-1), 4.56 (1 H, d, J 12.0 Hz, OCH_2Ph), 4.47 (1 H, d, OCH_2Ph), 3.93 (1 H, dq, J_{4-5}≈J_{5-6} 6.3 Hz, H-5), 3.63 (1 H, ddd, J_{3eq-4} 4.5, J_{3ax-4} 7.5 Hz, H-4), 2.99 (1 H, ddd, J_{gem} 15.3 Hz, H-3eq), 2,51 (1 H, dd, H-3ax), 1.40 (3 H, d, H-6) ESMS Calcd. for $C_{14}H_{18}O_4Na$ 273.1 Found 273.0.

*Methyl 4-O-benzyl-3,6 dideoxy-β-D-*arabino-*hexopyranoside* (**33**).

Uloside **32** (20 mg, 0.08 mmol) was dissolved in dry THF and cooled to –78 °C. L-Selectride (1M in THF)(500 μL) was added and the reaction was allowed to warm to room temperature. The reaction was then quenched with the addition of methanol and extracted with dichloromethane. After drying and concentration column chromatography in hexane:EtOAc (3:1) gave a colourless oil (14 mg, 71 %). ^{1}H NMR (300 MHz, $CHCl_3$): δ 7.22-7.34 (5 H, m, Ar), 4.75 (1 H, s, H-1), 4.51 (1 H, d, J 12.0 Hz, OCH_2Ph), 4.34 (1 H, d, OCH_2Ph), 3.98 (1 H, dd, J_{2-3eq} 3.6, J_{2-3ax} 3.0, H-2), 3.52 (3 H, s, OMe) 3.49-3.42 (2 H, m, H-4, H-5), 2.44 (1 H, ddd, J_{3-4} 3.6, J_{gem} 13.5 Hz, H-3eq), 1.51 (1 H, ddd, J_{3-4} 10.5 Hz, H3ax), 1.32 (3 H, d, J_{5-6} 5.7 Hz, H-6) ESMS Calcd. for $C_{14}H_{20}O_4Na$ 275.1 found 275.1

p-*Methoxyphenyl 4,6-O-benzylidene-β-D-glucopyranoside* (**34**).

p-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (8.0g 17 mmol) was dissolved in dry methanol (50 mL) and a catalytic amount of sodium metal (~10 mg) was added to the reaction. After stirring for 2 h TLC in 1:1 EtOAc:hexane showed complete conversion to baseline material. The solution was then neutralized with AG 50W H^+ resin and filtered. Concentration to dryness gave a white solid to which dry acetonitrile (100mL) was added followed by benzaldehyde dimethylacetal (4.0 mL, 26 mmol). The mixture was then heated to reflux and *p*TSA (200 mg) was added. Once a homogeneous solution was obtained heating was terminated and the solution was neutralized with triethylamine. The reaction mixture was then concentrated to dryness to give a white solid. Recrystallization from methanol gave white needles 5.46 g (86%); m.p. 213-214 °C; $[\alpha]_D$ -35.0° (*c* 1.0, DMF); ^{1}H NMR (300 MHz, CD_3OD): δ 7.48-7.51 (m, 2 H, Ar), 7.32-7.35 (m, 3 H, Ar), 6.99-7.04 (m, 2 H, Ar), 6.82-6.86 (m, 2 H, Ar), 5.59 (s, 1 H, PhC<u>H</u>O_2), 4.91 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.20 (dd, 1 H, J_{gem} 10.1, $J_{5,6eq}$ 3.8 Hz, H-6eq), 3.78 (dd, 1 H, $J_{5,6ax}$ 8.25 Hz, H-6ax), 3.74 (s, 3 H, OCH_3), 3.72 (dd, 1 H, $J_{2,3}$≈$J_{3,4}$ 9.0 Hz, H-3), 3.49-3.57 (3 H, m, H-2, H-5, H-4); Anal. Calcd.. for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.14; H, 5.79.

p-*Methoxyphenyl 4,6-O-benzylidene-2,3-di-O-chlorosulphate-β-D-glucopyranoside* (**35**).

A solution of **34** (4.7 g, 12.7 mmol) in dry dichloromethane (50 mL) and dry pyridine (25 mL) was cooled to -78°C. Sulfuryl chloride (2.25 mL, 28.0 mmol) was then added dropwise with stirring over 15 min and the solution was allowed to warm to room temperature over 2 h. The reaction was diluted with dichloromethane and washed with 10% H_2SO_4, followed by water, and dried over Na_2SO_4, and concentrated to a white solid. Compound **4** was crystallized from EtOAc-hexane (6.56g, 91%): m.p. 148°C decomp; $[\alpha]_D$ -63.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz CD_2Cl_2) δ 7.47-7.50 (m, 3 H, Ar), 7.36-7.40 (m, 2 H, Ar), 7.02-7.05 (m, 2 H, Ar), 6.85-6.88 (m, 2 H, Ar), 5.66 (s, 1 H, PhC<u>H</u>O_2), 5.14-5.29 (m, 3H, H-1, H-2, H-3), 4.48 (dd, 1 H, J_{gem} 10.6, $J_{5,6eq}$ 4.9 Hz, H-6_{eq}), 4.04 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.4 Hz, H-4), 3.90 (dd, 1 H, $J_{5,6ax}$ 10.0 Hz, H-6_{ax}), 3.77 (s, 3 H, OCH_3), 3.68 (ddd, 1 H, H-5); Anal. Calcd.. for $C_{20}H_{26}Cl_2O_{11}S_2$: C, 42.04; H, 3.53; Cl, 12.41. Found C, 41.88; H, 3.28; Cl, 12.69.

p-*Methoxyphenyl 4,6-O-benzylidene-3-bromo-3-deoxy-β-D-allopyranoside* (**36**).

To a solution of **35** (10.9 g, 19.1 mmol) in dry dichloromethane (75 mL) was added tetrabutylammonium bromide (10.9 g, 42.4 mmol) and the reaction was stirred for 1 h at room

temperature. The solution was diluted with dichloromethane and washed with saturated sodium bicarbonate, the organic solution was dried over sodium sulphate and concentrated to yellow syrup. The syrup was dissolved in methanol (200 mL) and water (5 mL), $KHCO_3$ (20 g) and KI (100 mg) were added. Stirring was continued for 15 min and then the solution was filtered. Concentration of the solution to syrup and chromatography in hexane:EtOAc (2:1) gave a colourless oil (8.01g, 96%); $[\alpha]_D$ -18.0°(*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz CD_2Cl_2) δ 7.49-7.52 (m, 3 H, Ar), 7.38-7.41 (m, 2 H, Ar), 7.38-6.99 (m, 2 H, Ar), 6.88-6.83 (m, 2 H, Ar), 5.65 (s, 1 H, PhC<u>H</u>O_2), 5.22 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.89 (dd, 1 H, $J_{2,3}$≈$J_{3,4}$ 3.4 Hz, H-3), 4.39 (dd, 1 H, J_{gem} 10.4, $J_{5,6eq}$ 5.1 Hz, H-6_{eq}), 4.15 (ddd, 1 H, $J_{4,5}$≈$J_{5,6ax}$ 9.7 Hz, H-5), 3.76-3.88 (m, 3 H, H-2, H-4, H-6ax), 3.71 (s, 3 H, OCH_3); Anal. Calcd.. for $C_{20}H_{21}O_6Br$: C, 54.93; H, 4.84. Found: C, 54.39; H, 4.83 ES HRMS $C_{20}H_{21}O_6BrNa$ 459.041919 $(M+Na)^+$, Found 459.041886.

p-Methoxyphenyl 4,6-O-benzylidene-3-bromo-2-O-tert-butyldimethylsilyl-3-deoxy-β-D-allo-hexopyranoside (**37**).

To a solution of **36** (2.6 g, 5.6 mmol) in dry DMF (20 mL), was added imidazole (600 mg, 8.81 mmol) and *t*-butyldimethylsilyl chloride (1.2 g, 8.0 mmol). The solution was heated to 60 °C and stirred for 3 h. After cooling the reaction was diluted with dichloromethane (150 mL) and washed with water. The organic phase was dried over sodium sulphate and concentrated to a syrup. Column chromatography in hexane:Et_2O (10:1), gave a colourless syrup (3.2 g 91%). $[\alpha]_D$ -41.8 (*c* 1.1, $CHCl_3$); ^1H NMR (300 MHz $CDCl_3$) δ 7.48-7.50 (m, 2 H, Ar), 7.36-7.34 (m, 3 H, Ar), 6.94-6.98 (m, 2 H, Ar), 6.80-6.83 (m, 2 H, Ar), 5.59 (s, 1 H, PhC<u>H</u>O_2), 5.23 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.67 (dd, 1 H, $J_{2,3}$≈$J_{3,4}$ 3.0 Hz, H-3), 4.38 (dd, 1 H, $J_{5,6ax}$ 9.6, J_{gem} 10.3 Hz, H-6_{ax}), 4.20 (ddd, 1 H, $J_{4,5}$ 9.0, $J_{5\text{-}6eq}$ 5.2 Hz, H-5), 3.85 (dd, 1 H, H-2), 3.81 (dd, 1 H, H-6_{eq}), 3.71 (dd, 1 H, H-4), 3.70 (s, 3 H, OMe), 0.90 (9H, s, $(CH_3)_3CSi$), 0.16 (s, 3 H, C<u>H</u>$_3$Si), 0.13 (s, 3H, C<u>H</u>$_3$Si) Anal. Calcd.. for $C_{26}H_{35}BrO_6Si$: C, 56.62; H, 6.40. Found: C, 56.93; H, 6.50.

p-*Methoxyphenyl 4,6-O-benzylidene-2-O-tert-butyldimethylsilyl-3-deoxy-β-D-ribo-hexopyranoside* (**38**).

The *allo*-hexopyranoside **37** (5.00 g, 10.5 mmol) was dissolved in a 1:1 mixture of EtOAc:EtOH, $KHCO_3$ (3 g) and 10% Pd/carbon (500 mg) of were added. The mixture was then stirred under 1 atm of hydrogen for 4 days, filtered through celite and concentrated to dryness. The resulting white solid was taken up in dichloromethane (200 mL) and washed with water, the organics were dried and concentrated. The resulting white solid was recrystallized from EtOAc-

hexane (4.32 g, 87%): $[\alpha]_D$ -63.6° (*c* 0.55, $CHCl_3$); m.p. 143 °C; ^{1}H NMR (500 MHz $CDCl_3$) δ 7.46-7.48 (m, 2 H, Ph), 7.32-7.37 (m, 3 H, Ph), 6.93-6.98 (m, 2 H, Ar), 6.81-6.83 (m, 2 H, Ar), 5.60(s, 1 H, $PhCHO_2$), 4.83 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.31 (dd, 1 H, J_{gem} 10.6, $J_{5,6eq}$ 4.3 Hz, H-6eq), 3.84 (ddd, 1 H, $J_{2,3eq}$ 5.2, $J_{2,3ax}$ 11.5 Hz, H-2), 3.75 (dd, 1 H, $J_{5,6ax}$ 10.6 Hz, H-6_{ax}), 3.75 (s 3 H, CH_3O), 3.62 (ddd, 1 H, $J_{3ax,4}$ 11.7, $J_{3eq,4}$ 2.3, $J_{4,5}$ 9.0 Hz, H-4), 3.50 (ddd, 1 H, H-5), 2.43 (ddd, 1 H, J_{gem} 12.2 Hz, H-3_{eq}), 1.84 (ddd, 1 H, H-3_{ax}), 0.87 (s, 9 H, $(C\underline{H}_3)_3CSi$), 0.12 (s, 3 H, $SiC\underline{H}_3$), 0.09 (s, 3H, $SiC\underline{H}_3$); Anal. Calcd. for $C_{26}H_{36}O_6Si$: C, 66.07;H, 7.68. Found: C, 65.73; H, 7.72 ES HRMS Calcd. for $C_{26}H_{36}O_6SiNa$ 495.217887 Found 495.217680.

p-*Methoxyphenyl* 4-O-*benzoyl-6-bromo-2*-tert-*butyldimethylsilyl-3,6-dideoxy*-β-D-ribo-*hexopyranoside* (**39**).

The 3-deoxy*ribo*hexopyranoside (**38**) (2.5 g, 5.29 mmol) was refluxed with *N*-bromosuccinimide (1.04 g, 5.84 mmol) and barium carbonate (0.63 g, 3.1 mmol) in carbon tetrachloride (50 mL) for 30 min. During this time the solution changed from colourless to deep orange and then to a pale yellow solution. The reaction was filtered, diluted with dichloromethane, washed with 5% sodium thiosulphate followed by water, and dried over sodium sulphate. The solution was then concentrated to a syrup and chromatographed in hexane:EtOAc (4:1) to give the 6-bromo-derivative (**39**) that crystallized upon standing (2.72 g 93%); $[\alpha]_D$ -40.7 ° (*c* 0.5, $CHCl_3$); m.p. 108-110° C; ^{1}H NMR (300 MHz $CDCl_3$) δ 7.99-8.02 (m, 2 H, Ph), 7.55-7.61 (m 1 H, Ar), 7.42-7.47 (m, 2 H, Ar), 7.04-7.08 (m, 2 H, PhOMe), 6.81-6.85 (m, 2 H, PhOMe), 4.98 (ddd, 1 H, $J_{3ax,4}$ 11.1, $J_{3eq,4}$ 4.8, $J_{4,5}$ 9.4 Hz, H-4), 4.80 (d, 1 H, J_{1-2} 7.3 Hz, H-1), 3.86-3.95 (m, 2 H, H-2, H-5), 3.57 (dd, 1 H, $J_{5,6}$ 2.5, J_{gem} 11.1 Hz, H-6a), 3.76 (s, 3 H, OCH_3), 3.41 (dd, 1 H, $J_{5,6}$ 8.2 Hz, H-6b), 2.56 (ddd, 1 H, J_{gem} 12.5, $J_{2,3eq}$ 5.1 Hz, H-3_{eq}), 1.79 (ddd, 1H, J_{2-3} 12.5 Hz, H-3_{ax}), 0.86 (s, 9 H, $(C\underline{H}_3)_3CSi$), 0.13 (s, 3 H, $SiC\underline{H}_3$), 0.12 (s, 3 H, $SiC\underline{H}_3$); Anal. Calcd.. for $C_{26}H_{35}BrO_6Si$: C, 56.62; H, 6.40. Found: C, 56.40; H, 6.30.

p-*Methoxyphenyl* 2-O-tert-*butyldimethylsilyl-3,6-dideoxy-β-D*-ribo-*hexopyranoside* (**40**).

To a solution of 6-bromo derivative (**39**) (1.0 g, 1.81 mmol) in ethanol was added $KHCO_3$ (500 mg) followed by 10% Pd/carbon (100 mg). The mixture was hydrogenated with stirring for 48 h at 1 atm. The mixture was filtered through celite and concentrated to a syrup. The syrup was taken up in EtOAc and washed twice with water. The organic phase was dried over sodium sulphate, concentrated to colourless syrup and chromatographed in EtOAc:hexane (2:1) to give a colourless oil (0.62g, 93%): $[\alpha]_D$ -51.9° (*c* 1.6, $CHCl_3$); ^{1}H NMR (300 MHz

$CDCl_3$) δ 6.92-6.96 (m, 2 H, Ar), 6.78-6.82 (m, 2 H, Ar), 4.77 (d, 1 H, $J_{1,2}$ 6.7 Hz, H-1), 3.78 (ddd, 1 H, $J_{2,3ax}$ 10.3, $J_{2,3eq}$ 5.0 Hz, H-2), 3.75 (s, 3 H, CH_3O), 3.40-3.48 (m, 2 H, H-4, H-5), 2.32 (ddd, 1 H, J_{gem} 12.6, $J_{3,4}$ 4.6 Hz, H-3_{eq}), 1.63 (ddd, 1 H, $J_{3ax,4}$ 10.3 Hz, H-3_{ax}), 1.30 (d, 3H, $J_{5,6}$ 5.9 Hz, H-6), 0.88 (s, 9 H, $(C\underline{H}_3)_3CSi$), 0.12 (s, 3 H, $SiC\underline{H}_3$), 0.09 (s, 3H, $SiC\underline{H}_3$). Anal. Calcd.. for $C_{19}H_{32}O_5Si$: C, 61.92; H, 8.75. Found: C, 61.61, H, 8.69; ES HRMS Calcd. for $C_{19}H_{32}O_5Si$ Na 391.191673 Found 391.191592.

p-*Methoxyphenyl 4-O-benzyl-2-O-tert-butyldimethylsilyl-3,6-dideoxy-β-D-ribo-hexopyranoside* (**41**).

To a solution of (**40**) (600 mg, 1.63 mmol) in dry DMF (10 mL) at room temperature was added benzyl bromide (420 μL, 3.53 mmol) followed by sodium hydride (65 mg, 2.7 mmol). After 5 h TLC indicated the absence of starting material. The solution was diluted with dichloromethane and washed with water. The organic layer was dried over sodium sulphate and concentrated. The resulting syrup was chromatographed in hexane:Et_2O (9:1) which gave a white solid (658 mg, 88%) that could be recrystallized from EtOAc-hexane mixtures: m.p. 59-60° C; [α]$_D$ -25.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz $CDCl_3$) δ 7.29-7.34 (m, 5 H, Ph), 6.93-6.96 (m, 2 H, Ar), 6.78-6.81 (m, 2 H, Ar), 4.70 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1,), 4.64 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.48 (d, 1 H, $PhC\underline{H}_2$), 3.75 (s, 3 H, CH_3O), 3.69 (ddd, 1 H, $J_{2,3eq}$ 5.2, $J_{2,3ax}$ 11.4 Hz, H-2), 3.50 (dq, 1 H, $J_{4,5}$ 9.0, $J_{5,6}$ 6.1 Hz, H-5), 3.17 (ddd, 1 H, $J_{3eq,4}$ 4.5, $J_{3ax,4}$ 11.2 Hz, H-4), 2.40 (ddd, 1 H, J_{gem} 12.6, H-3_{eq}), 1.56 (ddd, 1 H, H-3_{ax}), 1.31 (d, 3 H, H-6), 0.87 (s, 9 H,$(C\underline{H}_3)_3CSi$), 0.12 (s, 3 H, $SiC\underline{H}_3$), 0.08 (s, 3 H, $SiC\underline{H}_3$); Anal. Calcd.. for $C_{26}H_{38}0_5Si$: C, 68.08; H, 8.35. Found: C, 68.00; H, 8.58.

p-*Methoxyphenyl 4-O-benzyl-3,6-dideoxy-β-D-ribo-hexopyranoside* (**42**).

The paratose derivative (**41**) (532 mg, 1.21 mmol) was dissolved in 1M tetrabutylammmonium fluoride in THF (4 mL) and stirred at room temperature for 1 h. The solution was then concentrated and subjected to chromatography EtOAc:hexane (1:1). A white solid (385 mg, 92%) was obtained that could be recrystallized from EtOAc-hexane; m.p. 132° C. [α]$_D$ -25.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz $CDCl_3$) δ 7.29-7.34 (m, 5H, Ar), 6.93-6.96 (m, 2 H, Ar), 6.78-6.81 (m, 2 H, Ar), 4.78 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1), 4.64 (d, 1 H, J_{gem} 11.5 Hz, $PhC\underline{H}_2$), 4.50 (d, 1 H, $PhC\underline{H}_2$), 3.76 (s, 3 H, $C\underline{H}_3O$), 3.68 (m, 1 H, H-2), 3.61 (dq, 1 H, $J_{4,5}$ 8.2, $J_{5,6}$ 6.2 Hz, H-5), 3.25 (ddd, 1 H, $J_{3ax,4}$ 10.6, $J_{3eq,4}$ 4.3, H-4), 2.56 (ddd, 1 H, J_{gem} 12.5, $J_{2,3eq}$ Hz, H-3_{eq}), 1.60

(ddd, 1 H, $J_{2,3ax}$ 12.5 Hz, H-3ax), 1.32 (d, 3 H, H-6); Anal. Calcd.. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.55; H, 7.20.

p-*Methoxyphenyl 4-O-benzyl-3,6-dideoxy-2-O-pivaloyl-β-D-ribo-hexopyranoside* (**43**).

42 (300 mg, 0.87 mmol) was dissolved in pyridine (5 mL). DMAP (25 mg) and pivaloyl chloride (214 μL, 1.7 mmol) were added and the reaction was stirred at room temperature overnight. The reaction mixture was then concentrated and the residue dissolved in dichloromethane. The yellow solution was washed with water, dried over sodium sulphate, concentrated and chromatographed in hexane:Et_2O (7:1). The colourless syrup cystallized on standing (338 mg, 91%) and was recrystallized from EtOAc-hexane: m.p. 82-83°C, $[\alpha]_D$ -16.5° (c 1.0, $CHCl_3$); ^1H NMR (300 MHz $CDCl_3$) δ 7.25-7.36 (m, 5 H, Ar), 6.91-6.96 (m, 2 H, Ar), 6.76-6.82 (m, 2 H, Ar), 4.82-4.91 (ABX, 2 H, H-1, H-2), 4.62 (d, 1 H, J_{gem} 11.4 Hz PhC$\underline{H}_2$), 4.45 (d, 1 H, Hz PhC$\underline{H}_2$), 3.74 (s, 3 H, CH_3O), 3.56 (dq, 1 H, $J_{4,5}$ 8.9 Hz, $J_{5,6}$ 6.1 Hz, H-5), 3.28 (ddd, 1 H, $J_{3ax,4}$ 11.0, $J_{3eq,4}$ 4.6, H-4), 2.56 (ddd, 1 H, J_{gem} 12.5, $J_{2,3eq}$ 4.6 Hz, H-3_{eq}), 1.51 (ddd, 1 H, $J_{2,3ax}$ 11.0 Hz, H-3_{ax}), 1.34 (d, 3 H, H-6), 1.19 (s, 9 H, C(C$\underline{H}_3$)$_3$) Anal Calcd.. for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 69.86; H, 7.66.

Phenyl 4-O-benzyl-3,6-dideoxy-2-O-pivaloyl-1-thio-β-D-ribo-hexopyranoside (**44**).

To an ice cold solution of **43** (510 mg, 1.19 mmol) in dry dichloromethane was added thiophenol (196 μL, 1.8 mmol) followed by $BF_3{\cdot}OEt_2$ (200 μL, 1.43 mmol). The reaction was stirred for 2 h at 0 °C and then quenched with triethylamine. The resulting solution was diluted with dichloromethane and washed with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulphate and concentrated to an oil. Column chromatography of this oil in hexane:EtOAc (7:1) to gave a colourless syrup (445 mg, 90%). NMR revealed a 2:1 mixture of α :β anomers. Crystallization from hexane-EtOAc gave the pure α isomer. Data for the α anomer: mp 131 °C; $[\alpha]_D$ +272.2° (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz $CDCl_3$) δ 7.20-7.43 (m, 10 H, Ar), 5.64 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1), 4.97 (ddd, 1 H, $J_{2,3eq}$ 4.8, $J_{2,3ax}$ 12.5 Hz, H-2), 4.66 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$), 4.48 (d, 1 H, PhC$\underline{H}_2$), 4.21 (dq, 1 H, $J_{4,5}$ 9.3, $J_{5,6}$ 6.2 Hz, H-5), 3.22 (ddd, 1 H, $J_{3ax,4}$ 11.9, $J_{3eq,4}$ 4.5 Hz, H-4), 2.36 (ddd, 1 H, J_{gem} 11.9, H-3_{eq}), 1.83 (ddd, 1 H, H-3_{ax}), 1.25 (d, 3 H, H-6), 1.22 (s, 9H, C(C$\underline{H}_3$)$_3$); Anal Calcd.. for $C_{24}H_{30}O_4S$: C, 69.53; H, 7.29. Found: C 69.33; H, 7.33.

6-(Chloro)hexyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**45**).

3,4,6-Triacetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide (1 g, 2.0 mmol) was dissolved in dry dichloromethane (20 mL) and lutidine (200 μL, 1.7 mmol), 6-chloro-1-hexanol (3.1 mmol, 1.6 eq) and freshly dried 4A molecular sieves were added. The mixture was stirred for 2 h under argon and then cooled in an ice bath. Silver triflate (565 mg, 2.2 mmol) was added and the reaction was stirred in the dark for 3 h. The mixture was neutralized with triethylamine (2 mL) and filtered through celite. The solution was concentrated and chromatographed in EtOAc:pentane (2:1) to yield a colourless oil (997 mg, 90%); $[\alpha]_D$ 19.0° (*c* 1.7, $CHCl_3$); ^{1}H NMR (300 MHz $CDCl_3$) δ 7.79-7.84 (m, 2 H, Ar), 7.70-7.74 (m, 2 H, Ar), 5.75 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 9.0 Hz, H-3), 5.31 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.13 (dd, 1 H, $J_{4,5}$ 9.0 Hz, H-4), 4.29 (dd, 1 H, $J_{5,6}$ 4.6, J_{gem} 12.2 Hz, H-6a), 4.27 (dd, 1 H, H-2), 4.13 (dd, 1 H, $J_{5,6}$ 2.4 Hz, H-6b), 3.82 (m, 2 H, CH_2O, H-5), 3.39 (ddd, 1 H, J_{gem} 9.9, J_{vic} 7.2, J_{vic} 5.6 Hz, CH_2O), 3.27 (t, 2 H, J 6.9 Hz, CH_2Cl), 2.07 (s, 3 H, CH_3CO), 1.99 (s, 3 H, CH_3CO), 1.82 (s, 3 H, CH_3CO), 1.39 (m, 4 H, OCH_2CH_2, CH_2CH_2Cl), 1.11 (m, 4 H, $OCH_2CH_2CH_2CH_2$); Anal. Calcd.. for $C_{26}H_{32}NO_{10}$: C, 56.37; H, 5.82; N, 2.53. Found: C 56.08; H, 5.81; N 2.47.

6-(Chloro)hexyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**46**).

The triacetate **45** (1.0g, 1.8 mmol) was dissolved in dry methanol (25 mL) and a catalytic amount of sodium metal (~10 mg) was added. The reaction was stirred for 3 h and AG 50W H^+ resin was added until the solution was neutral on pH paper. The reaction was then filtered and concentrated. The resulting white solid was dissolved in dry acetonitrile (20 mL) and benzaldehyde dimethyl acetal (325 μL, 2.2 mmol) was added followed by *p*TSA (50 mg). The mixture was stirred for 20 min and then quenched by the addition of triethylamine. The reaction was concentrated and subjected to column chromatography in hexane:EtOAc (2:1) to give an amorphous solid (798 mg, 86%): $[\alpha]_D$ 35.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz $CDCl_3$) δ 7.79-7.84 (m, 2 H, Ar), 7.70-7.74 (m, 2 H, Ar), 7.44-7.46 (m, 2 H, Ar), 7.32-7.38 (m, 3 H, Ar), 5.55 (s, 1 H, $PhCHO_2$), 5.24 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.61 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3), 4.37 (dd, 1 H, J_{gem} 10.5, $J_{5,6}$ 4.5 Hz, H-6_{eq}), 4.22 (dd, 1 H, H-2), 3.78-3.85 (m, 2 H, CH_2O, H-5), 3.67-3.53 (m, 2 H, H-4, H-6_{ax}), 3.41 (ddd, 1 H, J_{gem} 9.7, J_{vic} 7.2, J_{vic} 5.9 Hz, CH_2O), 3.29 (t, 2 H, t, J 6.9 Hz, CH_2Cl), 1.33-1.48 (m, 4 H, OCH_2CH_2, CH_2CH_2Cl), 1.04-1.25 (m, 4 H, $OCH_2CH_2CH_2CH_2$); Anal. Calcd.. for $C_{23}H_{22}ClNO_7$: C, 60.07; H, 4.82; N, 3.05. Found: C 59.72; H, 4.67; N 2.93; ES HRMS Calcd. for $C_{27}H_{30}NO_7NaCl$ 538.16085 Found 538.161042.

6-(Chloro)hexyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**47**).

To a solution of (**46**) (2.5g, 4.8 mmol) in dry dimethylformamide (20 mL) was added benzyl bromide (700 µl, 5.8 mmol) followed by sodium hydride (150 mg, 6.2 mmol). The reaction was stirred overnight then diluted with dichloromethane and washed with water. The organic phase was dried over sodium sulphate, concentrated and subjected to column chromatography in hexane:EtOAc (3:1). The column yielded a white solid after concentration of the desired fractions which was recrystallized from EtOAc-hexane (2.6 g, 89%): m.p. 111° C; $[\alpha]_D$ 47.6.° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz $CDCl_3$) δ 7.6-7.9 (br m, 4 H, Ar), 7.49-7.52 (m, 2 H, Ar), 7.35-7.40 (m, 3 H, Ar), 6.84-7.00 (m, 5 H, Ar), 5.60 (s, 1 H, $PhC\underline{H}O_2$), 5.17 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.78 (d, 1 H, J_{gem}12.3 Hz, $C\underline{H}_2Ph$), 4.49 (d, 1 H, $C\underline{H}_2Ph$), 4.40 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 8.6 Hz, H-3), 4.38 (dd, 1 H, J_{gem} 10.4, $J_{5,6eq}$ 3.9 Hz, H-6_{eq}), 4.16 (dd, 1 H, H-2), 3.73-3.88 (m, 3 H, H-4, H-6_{ax}, $CH_2C\underline{H}_2O$), 3.62 (ddd, 1H, $J_{4,5}\approx J_{5,6ax}$ 9.7, H-5), 3.36 (dt, 1H, J_{gem} 8.7, J_{vic} 6.0 Hz, $CH_2C\underline{H}_2O$), 3.27 (t, 2 H, J 6.9 Hz, $C\underline{H}_2Cl$), 1.29-1.47 (m, 4 H, $OCH_2C\underline{H}_2$, $C\underline{H}_2CH_2Cl$), 0.98-1.20 (m, 4 H, $OCH_2CH_2C\underline{H}_2C\underline{H}_2$) Anal Calcd. for $C_{34}H_{38}NO_7$: C, 67.38; H, 5.99; N, 2.31. Found: C, 67.20; H, 6.04; N 2.28.

6-(Chloro)hexyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**48**).

The benzylidene acetal **47** (700 mg, 1.15 mmol) was added to a round bottom flask containing, 3A molecular sieves, methyl orange (2 mg) and sodium cyanoborohydride (360 mg, 5.73 mmol). The flask was purged with argon and dry THF (15 mL) was added. The mixture was allowed to stir for 2 h and then the flask was fitted with a dropping funnel containing a saturated solution of HCl in diethyl ether. This solution was added dropwise to the reaction mixture until the pink colour remained and no further gas evolution was evident. The reaction was stirred for 30 min then quenched with triethylamine, filtered through celite and concentrated. The resulting syrup was subjected to column chromatography, yielding a colourless oil (645 mg, 92%): $[\alpha]_D$ +18.3° (*c* 0.7, $CHCl_3$); ^1H NMR (300 MHz $CDCl_3$) δ 7.60-7.85 (br, 4 H, Ar), 7.27-7.38 (m, 5 H, Ar), 6.92-7.06 (m, 5 H, Ar), 5.11 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.73 (d, 1 H, J_{gem}12.2 Hz, $C\underline{H}_2Ph$), 4.60 (AB, 2 H, $OC\underline{H}_2Ph$), 4.52 (d, 1 H, $OC\underline{H}_2Ph$), 4.09-4.24(m, 2 H, H-2, H-3), 3.72-3.84 (m, 4 H, H-4, H-5, H-6a, $CH_2C\underline{H}_2O$), 3.62 (dd, 1 H, $J_{5,6b}$ 5.0, J_{gem} 9.8 Hz, H-6b), 3.36 (ddd, 1 H, J_{gem} 9.7, J_{vic} 7.1 J_{vic} 5.9 Hz $CH_2C\underline{H}_2O$), 3.26 (t, 2 H, J 6.9 Hz $C\underline{H}_2Cl$), 2.85 (s, 1 H, $J_{OH,4}$ 2.5 Hz, OH), 1.30-1.45 (m, 4 H, $OCH_2C\underline{H}_2$, $CH_2C\underline{H}_2Cl$), 0.99-1.20 (m, 4 H, $C\underline{H}_2C\underline{H}_2$); Anal Calcd. for $C_{34}H_{38}NO_7$: C, 67.15; H, 6.30; N, 2.30. Found: C 66.91; H, 6.46; N 2.27.

Phenyl 3,4,6-tri-O-acetyl-2-deoxy-1-thio-2-(2',2',2'-trichloroethoxycarbonylamino)-β-D-galactopyranoside (**49**).

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2',2',2'-trichloroethoxycarbonylamino)-β-D-galactopyranoside (1.23 g, 2.35 mmol) was dissolved in dichloromethane (20 mL). Thiophenol (480 μL, 4.62 mmol) and $BF_3{\cdot}OEt_2$ (390 μL, 3.0 mmol) were then added in succession under argon. After 3 h stirring at room temperature the reaction was diluted with dichloromethane and washed with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulfate and concentrated to a yellow oil that was subjected to column chromatography in hexane:EtOAc (2:1) to give a colourless oil (1.0 g 74%) that crystallized on standing. m.p. 117° C; $[\alpha]_D$ –5.8 (*c* 1.0, $CHCl_3$); (300 MHz $CDCl_3$) δ 7.53-7.48 (m, 2 H, Ar), 7.32-7.27 (m, 3 H, Ar), 5.38 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.18 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3), 5.01 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.89 (d, 1 H, $J_{NH,2}$ 10.4 Hz, NH), 4.73 (AB, 2 H, CH_2CCl_3), 4.17 (dd, 1 H, J_{gem} 11.4, $J_{5,6a}$ 7.1 Hz, H-6a), 4.11 (dd, 1 H, $J_{5,6}$ 6.1 Hz, H-6b), 3.91 (ddd, 1 H, H-5), 3.89 (ddd, 1H, H-2), 2.10 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO), 1.97 (s, 3 H, CH_3CO); Anal. Calcd. for $C_{21}H_{24}Cl_3NO_9S$: C, 44.03; H, 4.22; N, 2.45. Found: C, 44.12; H, 3.95; N, 2.45.

Methyl (3,4,6-tri-O-acetyl-2-deoxy-2-(2',2',2',-trichloroethoxycarbonylamino)-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**50**).

Methyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (664 mg, 1.16 mmol) and glycosyl donor **49** (530 mg 1.15 mmol) were added to a round bottom flask containing 4A molecular sieves. The flask was purged with argon and dichloromethane (5 mL) was added. The mixture was stirred for 1 h at rt and then *N*-iodosuccinimide (103 mg, 0.46 mmol) followed by silver triflate (20 mg, 0.078 mmol) were added to the reaction flask. Stirring was continued for 20 min and then the reaction was then diluted with dichloromethane and filtered through celite. The organic phase was washed with solutions of sodium bicarbonate and sodium thiosulfate, dried and concentrated to a yellow oil. Column chromatography in toluene:EtOAc (2:1) gave a white solid that was recrystallized EtOAc-hexane to give fine needles (967 mg, 87%): m.p. 83-85° C; $[\alpha]_D$ +2.5° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz $CDCl_3$) δ 7.32-7.76 (m, 9 H, Ar) 6.97 (m, 2 H, Ar), 6.78-6.86 (m, 3 H, Ar), 5.19 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4'), 4.97 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.92 (d, 1 H, J_{gem} 12.2 Hz, $PhC\underline{H}_2$), 4.79 (d, 1 H, J_{gem}12.4 Hz, $C\underline{H}_2CCl_3$), 4.76 (d, 1 H, J_{gem} 12.2 Hz, $PhC\underline{H}_2$), 4.66 (d, 1H, $C\underline{H}_2CCl_3$), 4.51 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-3'), 4.35 (d, 2H, $PhC\underline{H}_2O$, $PHC\underline{H}_2O$), 4.22 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1'), 4.19 (dd, 1 H, $J_{3,4}$ 8.4, $J_{2,3}$ 10.7 Hz, H-3), 4.10

(dd, 1 H, H-2), 3.99 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.96 (dd, 1 H, J_{gem} 11.1, $J_{5,6}$ 6.6 Hz, H-6a'), 3.85 (dd, 1 H, $J_{5,6}$ 7.3 Hz, H-6b'), 3.80 (dd, 1 H, dd, J_{gem} 10.6, $J_{5,6}$ 2.4 Hz, H-6a), 3.76 (m, 2 H, NH, H-2'), 3.64 (ddd, 1H, H-5'), 3.59 (1H, dd, $J_{5,6}$ 1.4 Hz H-6b), 3.46 (ddd, 1 H, H-5), 3.36 (s, 3 H, OMe), 2.02 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 1.95 (s, 3H, CH_3CO); Anal. Calcd.d. For $C_{44}H_{47}Cl_3N_2O_{16}$: C, 54.70; H, 4.90, N, 2.90. Found: C, 54.66; H, 4.84; N, 2.81.

6-(Chloro)hexyl (3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**51**).

The phenyl thioglycoside **49** (240 mg, 0.39 mmol) and acceptor **48** (230 mg 0.38 mmol) were added to a round bottom flask containing 4A molecular sieves and the flask was purged with argon. Dichloromethane (10 mL) was added and the mixture was stirred for 1 h at rt. *N*-iodosuccinimide (103 mg, 0.46 mmol) and silver triflate (20 mg, 0.078 mmol) were added to the reaction flask and stirring was continued for 20 min. The reaction was then diluted with dichloromethane and filtered through celite, the organic phase was washed with sodium bicarbonate and sodium thiosulfate solutions, dried over sodium sulfate and concentrated to a yellow oil. Column chromatography in toluene:EtOAc (2:1) gave a white solid that was recrystallized (EtOAc/hexane) to give needles (396 mg, 93%); m.p. 153° C; $[\alpha]_D$ -12.5° (*c* 0.52, $CHCl_3$); ^{1}H NMR (300 MHz $CDCl_3$) δ 7.53-7.78 (br, 4 H, Ar), 7.37-7.51 (m, 5 H, Ar), 6.95-6.97 (m, 2 H, Ar), 6.78-6.86 (m, 3 H, Ar), 5.20 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.9 Hz, H-4'), 5.03 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.90 (d, 1 H, J_{gem} 12.1 Hz, CH_2Ph), 4.78 (d, 1 H, J_{gem} 12.1 Hz, CH_2CCl_3), 4.76 (d, 1 H, CH_2Ph), 4.66 (d, 1 H, CH_2CCl_3), 4.53 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3'), 4.36 (d, 1 H, J_{gem} 12.4 Hz, CH_2Ph), 4.34 (d, 1 H, CH_2Ph), 4.24 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1'), 4.18 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 8.2 Hz, H-3), 4.10 (dd, 1 H, H-2), 3.98 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.94 (d, 1 H, J_{gem} 11.2 Hz, H-6a), 3.87 (dd, $J_{5,6b}$ 7.3 Hz, H-6b), 3.75-3.83 (m, 3 H, H-2', H-6a', CH_2CH_2O), 3.64 (ddd, 1 H, $J_{5,6}$ 6.9 Hz, H-5'), 3.58 (d, 1 H, J_{gem} 10.2Hz, H-6b'), 3.45 (d, 1 H, H-5), 3.32 (ddd, 1 H, J_{gem} 9.8, J_{vic} 5.8, J_{vic} 7.32 Hz, CH_2CH_2O), 3.35 (t, 2 H, CH_2Cl), 2.02 (s, 3 H, CH_3CO), 2.01 (s, 3 H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.29-1.42 (m, 4 H, OCH_2CH_2, CH_2CH_2Cl), 0.98-1.1.18 (m, 4 H, $OCH_2CH_2CH_2CH_2$); Anal Calcd. for $C_{49}H_{56}Cl_4N_2O_{16}$: C, 54.96; H, 5.23; N, 2.62. Found: C, 54.94; H, 5.16; N, 2.56.

Methyl 2-acetamido-(3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**52**).

To a solution of the disaccharide **50** (746 mg, 0.77 mmol) in 1:1 DMF:AcOH (20 mL) was added cadmium metal powder (490 mg, 11.5 mmol) and the reaction was stirred under argon

overnight. The reaction was then filtered and concentrated to yellow oil. The oil was taken up in methanol (20 mL) and treated with acetic anhydride (0.5 mL). The solution was stirred for 1 h then concentrated to a syrup. Column chromatography in EtOAc:hexane:methanol (10:4:1) yielded a white amorphous solid (474 mg 74%). $[\alpha]_D$ 9.6° (*c* 1.0 $CHCl_3$) ^1H NMR (500 MHz CD_3OD) δ 7.66-7.80 (m, 4 H, Ar), 7.27-7.45 (m, 5 H, Ar), 6.97-7.00 (m, 2 H, Ar), 6.58-6.87 (m, 3 H, Ar), 5.28 (dd, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ 1.7 Hz, H-4'), 5.08 (dd, 1 H, $J_{2,3}$ 11.3 Hz, H-3'), 5.03 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.85 (d, 1 H, J_{gem} 12.2 Hz, PhC$\underline{H}_2$O), 4.77 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1'), 4.72 (d, 1 H, J_{gem} 11.6 Hz, PhC$\underline{H}_2$O), 4.64 (d, 1H, PhC$\underline{H}_2$O), 4.44 (d, 1H, PhC$\underline{H}_2$O) 4.25 (dd, 1H, $J_{2,3}$ 10.7, $J_{3,4}$ 8.6 Hz, H-3), 4.03-4.10 (m, 4 H, H-2', H-4, H-6a', H-6b'), 3.97 (dd, 1 H, H-2), 3.86 (dd, 1 H, J_{gem}11.3, $J_{5,6}$ 1.8 Hz, H-6a), 3.81 (dd, 1 H, $J_{5,6}$ 3.8 Hz, H-6b), 3.78 (ddd, 1H, $J_{5,6a} \approx J_{5,6b}$ 6.7 Hz, H-5'), 3.60 (ddd, 1H, $J_{4,5}$ 9.9, H-5), 3.36 (s, 3 H, OMe), 2.30 (s, 3H, C$\underline{H}_3$CO), 1.99 (s, 3H, C$\underline{H}_3$CO), 1.96 (s, 3H, C$\underline{H}_3$CO), 1.94 (s, 3H, C$\underline{H}_3$NHCO); Anal. Calcd. for $C_{43}H_{48}N_2O_{15}$: C, 62.01; H, 5.81, N, 3.36. Found: C, 61.93; H, 5.65; N, 3.20.

*6-(Chloro)hexyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside* (**53**).

To a solution of **51** (2.2 g, 2.1 mmol) in 1:1 DMF:AcOH (30 mL) was added cadmium metal powder (1.3 g, 11.5 mmol) and the reaction was stirred under argon overnight. The reaction was then filtered and concentrated to yellow oil. The oil was taken up in methanol (20 mL) and acetic anhydride (1 mL) was added. The solution was stirred for 1 h and concentrated to yellow syrup. Column chromatography in EtOAc:hexane:methanol (10:4:1) yielded 1.7 g (86%) of white solid which was recrystallized from EtOAc-hexane: m.p. 150° C; $[\alpha]_D$ +2.0 ° (*c* 1.0, $CHCl_3$) ^1H NMR (500 MHz $CDCl_3$) δ7.56-7.76 (br, 4 H, Ar), 7.40-7.50 (m, 5 H, Ar), 6.99-7.00 (m, 2 H, Ar), 6.78-6.87 (m, 3 H, Ar), 5.21 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 5.04 (d, 1 H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.91 (d, 1 H, J_{gem} 12.1 Hz, C$\underline{H}_2$Ph), 4.77 (d, 1 H, J_{gem} 12.5 Hz, C$\underline{H}_2$Ph), 4.58 (dd, 1 H, dd, $J_{2,3}$ 11.3, $J_{3,4}$ 3.5 Hz, H-3'), 4.43 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1'), 4.39 (d, 1 H, $J_{NH,2}$ 8.4 Hz, N$\underline{H}$), 4.38 (d, 1 H, J_{gem} 12.5 Hz, C$\underline{H}_2$Ph), 4.38 (d, 1 H, J_{gem} 12.0 Hz, C$\underline{H}_2$Ph), 4.21 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 8.4 Hz, H-3), 4.10 (ddd, 1 H, H-2'), 4.10 (dd, 1 H, H-2), 3.98 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.97 (dd, 1 H, $J_{5,6}$ 6.4, J_{gem} 11.5 Hz, H-6a'), 3.89 (dd, 1 H, $J_{5,6}$ 6.4 Hz, H-6b'), 3.77 (dt, 1 H, J_{gem} 9.7, J_{vic} 6.1, $CH_2C\underline{H}_2O$), 3.68 (m, 2 H, H-5', H-6a), 3.62 (dd, 1 H, J_{gem} 10.8, $J_{5,6}$ 2.1 Hz, H-6b), 3.51 (ddd, 1 H, $J_{5,6a}$ 2.4 Hz, H-5), 3.31 (ddd, 1 H, J_{vic} 7.2, J_{vic} 6.0Hz, $CH_2C\underline{H}_2O$), 3.25 (t, 2 H, J 6.9 Hz, C$\underline{H}_2$Cl), 2.02 (s, 3 H, C$\underline{H}_3$CO), 1.95 (s, 3 H, C$\underline{H}_3$CO), 1.74 (s, 3 H, C$\underline{H}_3$CO), 1.29-1.43 (m 4 H, $OCH_2C\underline{H}_2$

$C\underline{H}_2CH_2Cl$), 0.86-1.16 (m, 4 H, $OCH_2CH_2C\underline{H}_2C\underline{H}_2$); Anal. Calcd. for $C_{48}H_{57}ClN_2O_{15}$: C, 61.50; H, 6.13: N, 2.99. Found: C, 61.25; H, 6.16; N, 2.93.

Methyl (2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**54**).

To a solution of **52** (300 mg, 0.36 mmol) in dry methanol (15 mL) was added a catalytic amount of sodium metal (5 mg). The reaction was stirred at room temperature for 3 h, neutralized with AG 50W 50 H^+ resin, filtered and concentrated to dryness. The white solid was taken up in dry acetonitrile (15 mL) and benzaldehyde dimethyl acetal (108 μL, 0.71 mmol) followed by *p*TSA (20 mg) were added. The reaction was stirred for 20 min, neutralized with triethylamine, concentrated and subjected to column chromatography in EtOAc:hexane:methanol (10:4:1) to yield a white solid (245 mg 88%). Recrystallization from EtOAc-hexane gave the title compound **54**; m.p. 123-125° C; $[\alpha]_D$ +3.3° (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz CD_3OD) δ 7.54-7.78 (br, 4 H, Ar), 7.32-7.44 (m, 6 H, Ar), 7.02-7.24 (m, 5 H, Ar), 6.94-6.98 (m, 2 H, Ar), 6.72-6.76 (m, 2 H, Ar), 5.84 (d, 1 H, $J_{NH,2}$ 6.8, N$\underline{H}$), 5.47 (s, 1 H, C$\underline{H}O_2$Ph), 4.89 (d, 1H, J_{gem} 12.8 Hz, PhC$\underline{H}_2$O), 4.99 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.79 (d, 1 H, J_{gem} 11.7 Hz, PhC$\underline{H}_2$O), 4.57 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1'), 4.55 (d, 1 H, J_{gem} 12.1 Hz, PhC$\underline{H}_2$O), 4.51 (d, 1 H, J_{gem} 12.7 Hz, PhC$\underline{H}_2$O), 4.29 (dd, 1 H, $J_{2,3}$ 8.2, $J_{3,4}$ 10.7 Hz, H-3), 4.24 (d, 1 H, J_{gem}12.4 Hz, H-6a'), 4.11 (dd, 1H, H-2), 4.09 (dd, 1 H, $J_{3,4}$ 3.5, H-4'), 4.01 (dd, 1 H, $J_{4,5}$ 9.3 Hz , H-4), 3.91 (dd, 1 H, H-6b'), 3.86 (dd, 1 H, J_{gem} 10.8, $J_{5,6}$ 3.6 Hz, H-6a), 3.75 (dd, 1 H, $J_{5,6}$ 3.6 Hz, H-6b), 3.64 (ddd, 1 H, H-5) 3.92 (ddd, 1 H, H-2'), 3.59 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-3'), 3.37 (s 3 H, OMe), 3.25 (s, 1 H, H-5'), 1.89 (s, 3 H, CH_3CONH); Anal Calcd. for $C_{44}H_{46}N_2O_{12}$+0.5 H_2O: C, 65.74; H, 5.89; N, 3.48. Found: C, 65.75; H, 5.76; N, 3.44; 72 ES HRMS Calcd. for: 817.29484 Found: 817.29467.

6-(Chloro)hexyl (2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**55**).

To a solution of **53** (1.3 g, 1.4 mmol) in dry methanol (25 mL) was added a small piece of sodium metal (5 mg). The reaction was stirred at room temperature for 3 h then quenched with Ag 50W 50 H^+ resin, filtered and concentrated to dryness. The white solid was then taken up in dry acetonitrile and benzaldehyde dimethyl acetal (320 μL, 2.1 mmol) followed by *p*TSA (10 mg) were added. The reaction was stirred for 20 min, neutralized with triethylamine, concentrated and subjected to column chromatography EtOAc:hexane:methanol (10:4:1). A white solid (993 mg 79%) was obtained which could be recrystallized from EtOAc-hexane: m.p. 102-

103 °C; $[\alpha]_D$ -4.1° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz $CDCl_3$) δ 7.56-7.76 (br, 4 H, Ar), 7.34-7.43 (m, 5 H, Ar), 7.16-7.21 (m, 5 H, Ar), 6.96-6.98 (m, 2 H, Ar), 6.72-6.86 (m, 3 H, Ar), 5.85 (d, 1 H, $J_{NH,2}$ 6.9 Hz, NHAc), 5.48 (s, 1 H, $PhCHO_2$), 5.06 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.91 (d, 1 H, J_{gem} 12.7 Hz, OCH_2Ph), 4.79 (d, 1 H, J_{gem} 11.8 Hz, OCH_2Ph), 4.56 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1'), 4.54 (d, 1 H, J_{gem} 12.1 Hz, OCH_2Ph), 4.52 (d, 1 H, d, J 12.8 Hz, OCH_2Ph), 4.28 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 8.7 Hz, H-3), 4.24 (d, 1 H, J_{gem} 12.4 Hz, H-6a'), 4.11 (dd, 1 H, H-2), 4.08 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4'), 3.97 (dd, 1 H $J_{4,5}$ 8.4 Hz, H-4), 3.90-3.94 (m, 2 H, H-2', H-6b'), 3.84 (dd, 1 H, $J_{5,6}$ 3.1, J_{gem} 10.8, H-6a), 3.76 (dt, 1 H, J_{gem} 9.8, J_{vic} 5.8 Hz, CH_2CH_2O), 3.74 (dd, 1H, $J_{5,6}$ 3.4 Hz, H-6b), 3.58 (dd, 1 H, H-5), 3.58 (dd, 1 H, $J_{2,3}$ 10.7, H-3'), 3.32 (dt, 1 H, J 6.1 Hz, CH_2CH_2O), 3.25 (d, 1 H, $J_{5,6b}$ 4.6 Hz, H-5'), 3.25 (t, 2 H, J 6.9, CH_2Cl), 1.88 (s, 3 H, CH_3CO), 1.29-1.43 (m, 4H, OCH_2CH_2, CH_2CH_2Cl), 0.97-1.16 (m, 4H CH_2CH_2), Anal. Calcd. for $C_{49}H_{55}ClN_2O_{12}$: C, 65.43; H, 6.16; N, 3.11. Found: C, 65.16; H, 6.1; N, 3.07.

6-(Azido)hexyl 2-acetamido-(4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**56**).

To a solution of **55** (993 mg, 1.10 mmol) in DMSO (10 mL) was added sodium azide (400 mg, 6.2 mmol). The mixture was heated to 60 °C overnight. The solution was then diluted with dichloromethane and washed three times with water. After drying the solution was concentrated and crystallised from EtOAc-hexane to give **56** (946 mg 96%): m.p. 108-111° C; $[\alpha]_D$ -5.4° (*c* 0.52, $CHCl_3$); ^1H NMR (500 MHz $CDCl_3$) δ 7.56-7.76 (br, 4 H, Ar), 7.34-7.43 (m, 5 H, Ar), 7.16-7.21 (m, 5 H, Ar), 6.96-6.98 (m, 2 H, Ar), 6.72-6.86 (m, 3 H, Ar), 5.85 (d, 1 H, $J_{NH,2}$ 6.9 Hz, NHAc), 5.48 (s, 1 H, $PhCHO_2$), 5.06 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.91 (d, 1 H, J_{gem} 12.7 Hz, CH_2Ph), 4.79 (d, 1 H, J_{gem} 11.8 Hz, CH_2Ph), 4.56 (d, 1 H, $J_{1,2}$ 8.7Hz, H-1'), 4.54 (d, 1 H, J_{gem} 12.1 Hz, CH_2Ph), 4.52 (d, 1 H, J_{gem} 12.8 Hz, CH_2Ph), 4.28 (dd, 1H, $J_{2,3}$ 8.7, $J_{3,4}$ 10.4 Hz, H-3), 4.24 (d, 1 H, J_{gem} 11.3 Hz, H-6a'), 4.11 (dd, 1 H, H-2), 4.04 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4'), 3.97 (dd, 1 H, Hz, H-4), 3.90-3.94 (m, 2 H, H-2', H-6b'), 3.84 (dd, 1 H, J_{gem} 10.8, $J_{5,6}$ 3.1 Hz, H-6a), 3.76 (dt, 1 H, J_{gem} 9.8, J_{vic} 5.8 Hz, CH_2CH_2O), 3.74 (dd, 1 H, $J_{5,6}$ 3.4 Hz, H-6b), 3.62 (ddd, 1 H, $J_{4,5}$ 9.6 Hz, H-5), 3.58 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3'), 3.32 (dt, 1 H, J_{vic} 5.6 Hz, CH_2CH_2O), 2.97 (t, 2 H, J 7.0 Hz, CH_2N_3), 3.25 (s, 1 H, H-5'), 1.88 (s, 3H, CH_3CO), 1.28-1.42 (m, 2 H, OCH_2CH_2), 1.14-1.23 (m, 2 H, $CH_2CH_2N_3$), 0.97-1.10 (m, 4 H, $OCH_2CH_2CH_2CH_2$); IR (cast) N_3 stretch 2094 cm^{-1}; Anal. Calcd. For $C_{49}H_{55}N_3O_{12}$: C, 64.96; H, 6.12: N, 7.73. Found: C, 64.73; H, 6.13; N, 7.65.

Methyl (4-O-benzyl-3,6-dideoxy-2-O-pivaloyl-β-D-ribo-hexopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**57**).

A solution of **54** (85 mg, 0.11 mmol), **44** (49 mg, 0.119 mmol) and freshly activated 4A sieves in dichloromethane (3 mL) was stirred for 1 h. The reaction was cooled in an ice bath and *N*-iodosuccinimide (29 mg, 129 mmol) followed by silver triflate (10 mg) were added. The ice bath was removed and the reaction was allowed to warm to room temperature. After 30 min the reaction was filtered through celite, solids were washed with dichloromethane. The combined filtrate was washed with thiosulphate and bicarbonate solutions, dried over sodium sulfate and concentrated. Chromatography in toluene:EtOAc (5:2) yielded a clear amorphous solid (95mg, 85%): $[\alpha]_D$ 4.2° (*c* 1.0, $CHCl_3$) ^1H (500 MHz $CDCl_3$) δ 7.56-7.76 (m, 4 H, Ar), 7.12-7.44 (m, 15 H, Ar), 6.91-6.93 (m, 2 H, Ar), 6.79-6.82 (m, 1 H, Ar), 6.71 (m, 2 H, Ar), 5.59 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.47 (s, 1 H, PhC$\underline{H}O_2$), 5.17 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1'), 5.00 (d, 1 H, J_{gem} 12.5 Hz, PhC$\underline{H}_2$O), 4.99 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.69 (d, 1 H, J_{gem} 11.8 Hz, PhC$\underline{H}_2$O), 4.65 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1''), 4.64 (d, 1 H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$O), 4.60 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$O), 4.58 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.8 Hz, H-3'), 4.57 (d, 1 H, J_{gem} 12.8 Hz PhC$\underline{H}_2$O), 4.54 (ddd, 1 H, $J_{2,3ax}$ 11.1, $J_{2,3eq}$ 5.2 Hz, H-2''), 4.42 (d, 1 H, J_{gem} 11.4 Hz, PhC$\underline{H}_2$O), 4.28 (dd, 1 H, J_{gem} 12.8, $J_{5,6a}$ 1.4 Hz, H-6a'), 4.26 (d, 1 H, H-4'), 4.20-4.24 (m, 2H, H-3, H-4), 4.01 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.96 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b'), 3.81 (dd, 1 H, J_{gem} 10.8, $J_{5,6a}$ 1.5 Hz, H-6a), 3.76 (dd, 1 H, $J_{5,6b}$ 3.7 Hz, H-6b), 3.59 (ddd, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.49 (dt, 1 H, $J_{4,5}$ 8.9, $J_{5,6}$ 6.3 Hz, H-5''), 3.48 (dd, 1 H, H-2'), 3.36 (s, 3 H, OMe), 3.29 (dd, 1 H, H-5'), 3.23 (ddd, 1 H, $J_{3ax,4}$ 10.7, $J_{3eq,4}$ 4.6 Hz, H-4''), 2.62 (ddd, 1 H, J_{gem} 12.1 Hz, H-3_{eq}''), 1.97 (s, 3 H, C$\underline{H}_3$CONH), 1.37 (ddd, 1 H, H-3_{ax}''), 1.33 (d, 3 H, H-6''), 1.20 (s, 9 H, (C$\underline{H}_3$)$_3$CO); Anal. Calcd. for $C_{62}H_{70}N_2O_{16}$: C, 67.74; H, 6.42; N, 2.55. Found: C, 66.98; H, 6.15; N, 2.65; 72 ES HRMS Calcd. for 1099.48036 found 1099.48045.

6-(Azido)hexyl (4-O-benzyl-3,6-dideoxy-2-O-pivaloyl-β-D-ribo-hexopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**58**).

A solution of **56** (200 mg, 0.22 mmol), **44** (100 mg, 0.24 mmol) and freshly activated 4A sieves in dichloromethane (4 mL) was stirred for 1 h. The reaction was then cooled in an ice bath and *N*-iodosuccinimde (65 mg) followed by silver triflate (10 mg) were added. The ice bath was removed and the reaction was allowed to warm to room temperature. After 30 min the reaction was filtered through celite and the solids were washed with dichloromethane. The combined

filtrate was washed with thiosulphate and bicarbonate solutions, dried over sodium sulfate and concentrated to a yellow oil. Chromatography in toluene:EtOAc (5:2) yielded colourless amorphous solid **58** (216 mg 81%); $[\alpha]_D$ -5.4° (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz $CDCl_3$) δ 7.80-7.54 (br, 4 H, Ar), 7.40-7.10 (m, 15 H, Ar), 6.94-6.93 (m, 2 H, Ar), 6.74-6.70 (m, 3 H, Ar), 5.56 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NHAc) 5.43 (s, 1 H, O_2CHPh), 5.14 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1'), 5.05 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.99 (d, 1 H, J_{gem} 12.8 Hz, OCH_2Ph), 4.68 (d, 1 H, J_{gem} 12.1 Hz, OCH_2Ph), 4.66 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1"), 4.62 (d, 1 H, J_{gem} 12.1 Hz, OCH_2Ph), 4.60-4.52 (m, 2 H, H-2", H-3'), 4.58 (d, 1 H, J_{gem} 11.5 Hz, OCH_2Ph), 4.53 (d, 1 H, J_{gem} 12.8 Hz, OCH_2Ph), 4.39 (d, 1 H, J_{gem} 11.5 Hz, OCH_2Ph), 4.28 (dd, 1 H, $J_{2,3}$ 8.2, $J_{3,4}$ 10.6 Hz, H-3), 4.26 (d, 1 H, J_{gem} 10.6 Hz, H-6a'), 4.20 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 4.15-4.12 (m, 2 H, H-4, H-2), 3.87 (d, 1 H, H-6b'), 3.80 (d, 1 H, J_{gem}9.9 Hz, H-6a), 3.76 (dt, 1 H, J_{gem} 9.9, J_{vic} 6.0 Hz, OCH_2CH_2), 3.72 (dd, 1 H, $J_{5,6}$ 4.2 Hz, H-6b), 3.59 (dd, 1 H, $J_{4,5}$ 10.6 Hz, H-5), 3.48 (dd, 1 H, $J_{2,3}$ 6.4 Hz, H-2'), 3.46 (dq, 1 H, $J_{5,6}$ 5.8 Hz, H-5"), 3.33 (dt, 1 H J_{vic} 7.1 Hz, OCH_2CH_2), 3.22 (s, 1 H, H-5'), 3.21 (1H, ddd, J_{3eq-4} 4.7, J_{3ax-4} 12.1 Hz, H-4"), 2.97 (t, 3 H, J_{vic} 7.1 Hz, CH_2N_3), 2.61 (1H, ddd, J_{gem} 12.1, $J_{2,3}$ 4.9 Hz H-3_{eq}"), 1.95 (s, 3 H, $NHCOCH_3$), 1.41-1.28 (m, 2 H, OCH_2CH_2), 1.33 (ddd, 1 H, $J_{2,3}$ 12.0 Hz, H-3_{ax}"), 1.31 (d, 3 H, H-6"), 1.25-1.17 (m, 2 H, $CH_2CH_2N_3$), 1.17 (s, 9 H, $COC(CH_3)_3$), 1.11-1.00 (m, 4 H, $OCH_2CH_2CH_2CH_2$); Anal. Calcd. For $C_{67}H_{79}N_5O_{16}+H_2O$: C, 65.51; H, 6.65: N, 5.70 Found: C, 65.51; H, 6.66; N, 5.68 ES HRMS for $C_{67}H_{80}N_5O_{16}$, 1210.5600, found 1210.5596.

Methyl (4-O-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**59**).

The protected trisaccharide **57** (95 mg 0.086 mmol) was dissolved in butanol (4 mL) and ethylenediamine (1 mL). The solution was heated to 110 °C for 16 h and then concentrated to yellow oil. The oil was taken up in methanol, and acetic anhydride (500 μL) was added. The mixture was stirred for 1 h and then concentrated to dryness. The oil was dissolved in dry methanol (10 mL) and sodium metal (10 mg) was added. The solution was refluxed for a further 12 h, neutralized with Ag 50W 50 H^+ resin, filtered and then concentrated to dryness. The resulting oil was chromatographed in EtOAc:hexane:methanol (10:4:1) yielding a white amorphous solid **59** (68 mg, 85%); $[\alpha]_D$ 6.6° (*c* 0.45, CH_3OH). ^1H (600 MHz CD_3OD) δ 7.18-7.44 (m, 20 H, Ar), 5.33 (s, 1 H, $PhCHO_2$), 5.16 (d, 1 H, J_{gem} 12.1, $PhCH_2O$), 4.74 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1'), 4.67 (d, 1 H, J_{gem} 11.7 Hz, $PhCH_2O$), 4.62 (d, 1 H, J_{gem} 11.5 Hz, $PhCH_2O$), 4.61 (d, 1 H, J_{gem} 11.9 Hz, $PhCH_2O$), 4.47 (d, 1 H, J_{gem} 11.5 Hz, $PhCH_2O$), 4.47 (d, 1 H, J_{gem} 11.5 Hz,

PhC$\underline{H}_2$O), 4.32 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.31 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1"), 4.26 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 4.14 (dd, 1 H, J_{gem} 12.1, $J_{5,6}$ 1.7 Hz, H-6a'), 4.10 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2'), 3.96 (dd, 1 H, $J_{5,6}$ 2.2 Hz, H-6b'), 3.95 (dd, 1 H, $J_{4,5}\approx J_{3,4}$ 8.5 Hz, H-4), 3.91 (dd, 1 H, dd, $J_{2,3}$ 11.0, $J_{3,4}$ 3.1 Hz, H-3'), 3.83 (dd, 1 H, J_{gem} 11.2, $J_{5,6}$ 2.4 Hz, H-6b), 3.81 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 3.79 (dd, 1H, $J_{5,6}$ 4.4 Hz, H-6a), 3.65 (dd, 1H, H-3), 3.51 (1H, ddd, H-5), 3.40 (dq, 1 H, $J_{4,5}$ 9.0, $J_{5,6}$ 6.0 Hz, H-5"), 3.34 (ddd, 1 H, $J_{2,3ax}$ 12.5 $J_{2,3eq}$ 7.7 Hz, H-2"), 3.43 (s, 3 H, OMe), 3.23 (s, 1 H, H-5'), 3.13 (ddd, 1 H, $J_{3ax,4}$ 11.0, $J_{3eq,4}$ 4.6 Hz, H-4"), 2.42 (1H, ddd, J_{gem} 12.0 Hz, H-3eq"), 1.94 (s, 3 H, C$\underline{H}_3$NHCO), 1.82 (s, 3 H, C$\underline{H}_3$NHCO), 1.33 (ddd, 1 H, H-3ax"), 1.27 (d, 3 H, H-6"); Anal. Calcd. for $C_{51}H_{62}N_2O_{14}+H_2O$: C, 64.82; H, 6.83; N, 2.96. Found: C, 64.83; H 6.64; N, 2.92; ES HRMS Calcd. for: $C_{51}H_{62}N_2O_{14}Na$ 949.4098. Found; 949.4088.

6-(Azido)hexyl (4-O-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**60**).

The protected trisaccharide **58** (176 mg, 0.145 mmol) was dissolved in butanol (4 mL) and ethenediamine (1 mL). The solution was heated to 110 °C for 12 h and then concentrated to a yellow oil. The oil was taken up in methanol and acetic anhydride (500 μL) was added, the mixture was stirred for 1 h. After concentration the resulting oil was dissolved in dry methanol (10 mL), sodium metal (10 mg) was added and the solution was refluxed for a further 12 h. It was then neutralized with Ag 50W 50 H^+ resin, filtered and concentrated. The remaining oil was chromatographed in EtOAc:hexane:methanol (10:4:1) yielding a white amorphous solid 132 mg 88%; $[\alpha]_D$ -5.4° (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz CD_3OD), δ 7.43-7.17 (m, 20 H, Ar), 5.52 (s, 1 H, $O_2C\underline{H}Ph$), 5.15 (d, 1 H, J_{gem} 11.9 Hz, OC$\underline{H}_2$Ph), 4.73 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1'), 4.68 (d, 1 H, J_{gem} 11.9 Hz, OC$\underline{H}_2$Ph), 4.67 (d, 1 H, J_{gem} 11.8 Hz, OC$\underline{H}_2$Ph), 4.62 (d, 1 H, J_{gem} 11.4 Hz, OC$\underline{H}_2$Ph), 4.60 (d, 1 H, J_{gem} 11.8 Hz, OC$\underline{H}_2$Ph), 4.47 (d, 1 H, J_{gem} 11.6 Hz, OC$\underline{H}_2$Ph), 4.40 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.30 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1"), 4.25 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4'), 4.14 (dd, 1 H, J_{gem} 12.2, $J_{5,6}$ 1.4 Hz, H-6a'), 4.09 (dd, $J_{2,3}$ 11.0 Hz, H-2'), 3.95 (dd, 1 H, $J_{5,6}$ 1.4 Hz, H-6'b), 3.94 (dd, 1 H, $J_{3,4}\approx J_{4,5}$ 8.5Hz, H-4), 3.90 (dd, 1 H, $J_{2,3}$ 11.1, $J_{3,4}$ 3.4 Hz, H-3'), 3.84-3.76 (m, 4 H, H-6a, H-6b, H-2, $CH_2C\underline{H}_2O$), 3.67 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-3), 3.50 (ddd, $J_{5\text{-}6a}$ 2.4, $J_{5\text{-}6b}$ 4.6 Hz, H-5), 3.45 (ddd, 1 H, J_{gem} 9.8 J_{vic} 6.4 Hz, $CH_2C\underline{H}_2O$), 3.40 (dq, 1 H, $J_{4,5}$ 9.0, $J_{5,6}$ 2.9 Hz, H-5"), 3.35 (ddd, 1H, $J_{2,3eq}$ 5.0, $J_{2,3ax}$ 12.2 Hz, H-2"), 3.23 (s, 1 H, H-5'), 3.23 (t, 2H, J_{vic} 7.0 Hz, CH_2N_3), 3.14 (ddd, 1 H, $J_{3,4}$ 4.6, $J_{3,4}$ 11.0 Hz, H-4"), 2.42 (ddd, 1 H, J_{gem} 12.1 Hz, H-3eq"), 1.94 (s, 3 H, NHCOC$\underline{H}_3$), 1.82 (s, 3 H, NHCOC$\underline{H}_3$), 1.58-1.52 (m, 4 H, $OCH_2C\underline{H}_2$, $C\underline{H}_2CH_2N_3$), 1.39-1.36 (m, 4 H,

$OCH_2CH_2C\underline{H}_2C\underline{H}_2$), 1.35 (ddd, 1 H, H-3ax"), 1.27 (d, 3 H, H-6"); Anal. Calcd. for $C_{56}H_{71}N_5O_{14}$+ H_2O: C, 63.68; H, 6.97: N, 6.63 Found C, 63.82; H, 6.94; N, 6.63.

*Methyl (4-O-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**61**).

The selectivitively deprotected trisaccharide **59** (75 mg) was dissolved in dry DMSO (2 mL) and acetic anhydride (0.5 mL). The reaction was stirred overnight and then concentrated to dryness. The yellow solid was dissolved in DMF (0.5 mL) and diluted with dry THF (10 mL). The reaction was cooled to –78° C and L-Selectride (1M in THF)(500 μL) was added dropwise. The reaction was allowed to warm to room temperature and was quenched with acetone. Silica gel was then added to the reaction and the volatiles were removed under vacuum. The silica gel was then slurried on the top of a silica column and the product was eluted with 4% methanol in dichloromethane to give **61** (62 mg 82%). $[\alpha]_D$ 4.3° (*c* 0.67 CH_3OH); ^1H NMR (500 MHz CD_3OD) δ 7.18-7.46 (m, 20 H, Ar), 5.17 (d, 1 H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$O), 5.55 (s, 1 H, PhC$\underline{H}O_2$), 4.74 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1'), 4.71 (d, 1 H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$O), 4.68 (d, 1 H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$O), 4.61 (d, 1 H, J_{gem} 11.9 Hz, PhC$\underline{H}_2$O), 4.58 (d, 1 H, $J_{1,2}$<1 Hz, H-1"), 4.57 (d, 1 H, J_{gem} 11.5 Hz, PhC$\underline{H}_2$O), 4.43 (d, 1 H, J_{gem} 11.5 Hz PhC$\underline{H}_2$O), 4.32 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.30 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 4.14 (dd, 1 H, J_{gem} 12.4, $J_{5,6}$ 1.4 Hz, H-6a'), 4.09 (dd, 1 H, $J_{2,3}$ 8.7 Hz, H-2'), 3.97 (dd, 1 H, $J_{5,6}$ 2.0 Hz, H-6b'), 3.95 (dd, 1 H, $J_{4,5}$ 6.9, $J_{3,4}$ 8.2 Hz, H-4), 3.93 (dd, 1 H, H-3'), 3.84 (dd, 1 H, J_{gem} 11.6, $J_{5,6a}$ 2.4 Hz, H-6a), 3.80 (dd, 1H, $J_{2,3}$ 9.7 Hz, H-2), 3.79 (dd, 1H, $J_{5,6b}$ 4.2 Hz, H-6b), 3.76 (ddd, 1 H, $J_{2,3a}$≈$J_{2,3b}$ 3.4 Hz, H-2") 3.66 (dd, 1 H, H-3), 3.51 (ddd, 1H, H-5), 3.43 (s, 3 H, OMe), 3.38-3.48 (m, 2 H, H-5", H-4"), 3.23 (s, 1 H, H-5'), 2.31 (ddd, 1 H, J_{gem} 13.5 Hz, H-3eq"), 1.94 (s, 3 H, C$\underline{H}_3$NHCO), 1.82 (s, 3 H, C$\underline{H}_3$NHCO), 1.48 (ddd, 1 H, H-3ax"), 1.30 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6"); ^{13}C NMR (500 MHz CD_3OD) δ 101.72 ($J_{C1\text{-}H1}$ 162 Hz, C-1', β), 103.20 ($J_{C1\text{-}H1}$ 161 Hz, C-1, β), 140.10 ($J_{C1\text{-}H1}$ 158 Hz, C-1", β); Anal. Calcd. for $C_{51}H_{62}N_2O_{14}$+H_2O: C, 64.82; H, 6.83; N, 2.96. Found: C, 64.77; H, 6.80; N, 2.95; ES HRMS Calcd. for $C_{51}H_{63}N_2O_{14}$ 927.4279. Found: 927.4294.

*6-(Azido)hexyl (4-O-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**62**).

The alcohol **60** (75 mg, 0.072 mmol) was dissolved in 2:1 dimethyl sulfoxide: acetic anhydride (6 mL) and stirred overnight under argon. The resulting yellow solution was concentrated to dryness and the yellow solid was taken up in a solution of DMF (0.5 mL) and tetrahydrofuran (10 mL). The solution was then cooled to –78 °C and treated with 20 equivalents of a 1M L-Selectride solution in THF (150 μL) added dropwise. The reaction was allowed to warm to –20 °C over 20 min, was quenched with ethylenediamine (1 mL) and allowed to warm to room temperature. Silica gel was added to the reaction and the volatiles were removed under vaccum. The silica was slurried on the top of a column and the product was eluted with 4% methanol in dichloromethane to give **62** (61 mg 81%) as an amorphous solid: $[\alpha]_D$ 8.9° (*c* 0.45, CH_3OH); ^1H NMR (500 MHz CD_3OD) δ 7.46-7.18 (m 20 H, Ar), 5.55 (s, 1 H, $O_2C\underline{H}Ph$), 5.17 (d, 1 H, J_{gem} 12.1 Hz, $OC\underline{H}_2Ph$), 4.74 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1'), 4.71 (d, 1 H, J_{gem} 11.7 Hz, $OC\underline{H}_2Ph$), 4.68 (d, 1 H J_{gem} 11.7 Hz, $OC\underline{H}_2Ph$), 4.61 (d, 1 H, J_{gem} 11.7 Hz, $OC\underline{H}_2Ph$), 4.59 (s, 1 H, H-1"), 4.58 (d, 1 H, J_{gem} 11.6 Hz, $OC\underline{H}_2Ph$), 4.43 (d, 1 H, J_{gem} 11.6 Hz, $OC\underline{H}_2Ph$), 4.41 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.30 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4'), 4.14 (dd, 1 H, J_{gem} 12.4, $J_{5,6}$ 1.4 Hz, H-6a'), 4.10 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-2'), 3.96 (dd, 1 H, $J_{5,6}$ 1.7 Hz, H-6b'), 3.94 (dd, 1 H, $J_{4,5}\approx J_{3,4}$ 8.6 Hz, H-4), 3.93 (dd, 1 H, H-3'), 3.86-3.78 (m, 3 H, H-6a, H-6b, $OC\underline{H}_2CH_2$), 3.81 (m, 1 H, H-2), 3.76 (dd, 1 H, $J_{2,3a}\approx J_{2,3b}$ 4.0 Hz, H-2"), 3.68 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-3), 3.51 (ddd, 1 H, $J_{5,6}$ 2.3, $J_{5,6}$ 4.6 Hz, H-5), 3.48-3.38 (m, 3 H, $OC\underline{H}_2CH_2$, H-4", H-5"), 3.23 (t, 2 H, J_{vic} 6.9 Hz, $C\underline{H}_2N_3$), 3.23 (s, 1 H, H-5'), 2.31 (ddd, 1 H, J_{gem} 13.6, $J_{3,4}$ 3.8 Hz, H-3eq") 1.94 (s 3 H, $C\underline{H}_3CONH$), 1.83 (s, 3 H, $C\underline{H}_3CONH$), 1.58-1.52 (m, 4 H, $OCH_2C\underline{H}_2CH_2CH_2C\underline{H}_2$), 1.48 (ddd, 1 H, $J_{3,4}$ 10.4 Hz, H-3ax"), 1.40 (m, 4 H, $OCH_2CH_2C\underline{H}_2C\underline{H}_2$), 1.30 (d, 3 H, $J_{5,6}$ 5.8 Hz, H-6"); Anal. Calcd. for $C_{56}H_{71}N_5O_{14}$+0.5 H_2O: C, 64.23; H, 6.93; N, 6.69. Found: C, 64.15; H, 6.58; N, 6.42; ES HRMS Calcd. for $C_{56}H_{72}N_5O_{14}$ 1038.5075 Found: 1038.5083.

Methyl (3,6-dideoxy-β-D-arabino-hexopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-2-acetamido- 2-deoxy-β-D-glucopyranoside (**1**).

The trisaccharide **61** (55mg) was hydrogenated for 16 h over 20% palladium hydroxide on carbon (10 mg) in methanol. After filtration the product was dissolved in a minimal amount of methanol and allowed to crystallize in an atmosphere of ethanol. The fine crystals were collected, dissolved in water and lyophilized to give **1** as a white solid (30 mg, 88%). $[\alpha]_D$ –26.9° (*c* 0.36, H_2O); ^1H NMR (600 MHz, D_2O) δ 4.70 (d, 1 H, $J_{1,2}$<1 Hz, H-1"), 4.58 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1'), 4.44 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.13 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4'), 4.04 (dd, 1H, $J_{2,3}$ 10.6 Hz, H-2'), 3.91 (dd, 1 H, $J_{2,3ax}\approx J_{2,3eq}$ 3.3 Hz, H-2"), 3.89 (dd, 1 H, $J_{3,4}$ 2.9 Hz, H-3'), 3.86 (dd, 1 H, J_{gem} 12.1,

$J_{5,6}$ 2.0 Hz, H-6b), 3.80 (dd, 1 H, J_{gem} 11.7, $J_{5,6}$ 8.2 Hz, H-6b'), 3.77 (dd, 1 H, dd, $J_{5,6}$ 7.3 Hz, H-6a'), 3.74 (dd, 1 H, H-5'), 3.71 (m, 2 H, H-2, H-3), 3.67 (dd, 1 H, $J_{5,6}$ 5.5 Hz, H-6a), 3.63 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 8.0 Hz, H-4), 3.55 (ddd, 1 H, $J_{3ax,4}$ 11.5, $J_{3eq,4}$ 4.7, J_{4-5} 9.3 Hz, H-4"), 3.53 (ddd, 1 H, H-5), 3.50 (s, 3 H, OMe), 3.44 (dq, 1 H, $J_{5,6}$ 6.2 Hz, H-5"), 2.17 (ddd, 1 H, J_{gem} 13.9 Hz, H-3b"), 2.06 (s, 3 H, $C\underline{H}_3NHCO$), 2.02 (s, 3 H, $C\underline{H}_3NHCO$), 1.66 (ddd, 1 H, H-3a"), 1.27 (d, 3 H, H-6") ^{13}C (600 MHz, D_2O) δ 18.2 (C-6"), 23.1 (CH_3NHCO), 23.2 (CH_3NHCO), 37.5 (C-3"), 52.4 (C-2'), 55.9 (C-2), 61.1 (C-6'), 61.8 (C-6'), 67.8 (C-4"), 68. 5 (C-2"), 68.8 (C-4'), 73.7 (C-3), 75.6 (C-5), 76.0 (C-5'), 76.5 (C-5"), 79.9 (C-3'), 80.3 (C-4), 102.8 (J_{C1-H1} 161.81 Hz, C-1, β), 102.2 (J_{C1-H1} 163.18 Hz C-1' β), 103.5 (J_{C1-H1} 159.76 C-1"β); ES HRMS Calcd. for $C_{23}H_{40}N_2O_{14}Na$: 591.2377. Found 591.2379.

6-(Amino)hexyl (3,6-dideoxy-β-D-arabino-hexopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-2-acetamido- 2-deoxy-β-D-glucopyranoside (**2**).

The protected trisaccharide **62** (30 mg) was dissolved in 3 mL ethylenediamine under argon. Lithium ribbon (10 mg) was added and the solution slowly turned deep blue. When the solution returned to yellow the reaction was quenched with methanol and the volatiles were removed under vacuum. The white solid obtained was dissolved in water and brought to neutral pH by the addition of 5M aqueous acetic acid as monitored on pH paper (1-12). The solution was then applied to a Sep Pak C18 reverse phase cartridge (Waters), washed with water and the crude product was eluted with 50% methanol. Further purification by HPLC on C18 silica (water + 0.1%TFA:methanol) gave pure **2** (14 mg 75% yield) as the trifluoroacetate salt; $[\alpha]_D$ −17.0 (*c* 0.27, H_2O): ^1H NMR (600 MHz, D_2O) 4.70 (d, 1 H, $J_{1,2}$<1 Hz, H-1"), 4.58 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1'), 4.49 (ABX, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.13 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4'), 4.04 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2'), 3.91-3.87 (m, 3 H, H-2", H-3', $OC\underline{H}_2CH_2$), 3.84 (dd, 1 H, J_{gem} 12.1, $J_{5,6}$ 2.0 Hz, H-6a), 3.80 (dd, 1 H, J_{gem} 11.7, $J_{5,6}$ 8.2 Hz, H-6a'), 3.76 (dd, 1H, $J_{5,6}$ 3.9 Hz, H-6b'), 3.74 (dd, 1 H, H-5'), 3.71 (ABX, 2 H, H-2, H-3), 3.65 (dd, 1 H, $J_{5,6}$ 5.7 Hz, H-6b), 3.63 (ABX, 1 H, H-4), 3.57 (dt, 1 H, J_{gem} 10.1, J_{vic} 6.4 Hz, $OC\underline{H}_2CH_2$), 3.55 (ddd, 1 H, $J_{3ax,4}$ 11.5, $J_{3eq,4}$ 4.8, $J_{4,5}$ 9.3 Hz, H-4"), 3.51 (ddd, 1 H, $J_{4,5}$ 7.7 Hz, H-5) 3.43 (dq, 1H, dq, $J_{5,6}$ 6.2 Hz, H-5"), 2.98 (t, 2 H, J_{vic} 7.7 Hz, $C\underline{H}_2NH_2$), 2.17 (ddd, 1 H, J_{gem} 13.9 Hz, H-3eq"), 2.06 (s, 3 H, $C\underline{H}_3CONH$), 2.20 (s, 3 H, $C\underline{H}_3CONH$), 1.65 (ddd, 1 H, H-3ax"), 1.65 (m, 2 H, $CH_2C\underline{H}_2CH_2NH_3$), 1.55 (m, 2 H, J 6.4, $CH_2C\underline{H}_2CH_2O$), 1.40-1.30 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), 1.27 (d, 3 H, H-6") ^{13}C (600 MHz, D_20) δ 17.9 (C-6"), 22.9 ($\underline{C}H_3NHCO$), 23.0 ($\underline{C}H_3NHCO$), 25.3, 25.97 ($OCH_2CH_2\underline{C}H_2$, $OCH_2CH_2CH_2\underline{C}H_2$) 27.41 ($\underline{C}H_2CH_2NH_3$), 29.09 ($OCH_2\underline{C}H_2$) 37.3 (C-3"), 52.6 (C-2'), 55.8 (C-2), 61.9 (C-6'), 67.7 (C-4"),

68.5 (C-2"), 68.8 (C-4'), 73.5 (C-3), 75.38 (C-5), 75.9 (C-5'), 76.9 (H-5"), 79.8 (C-3'), 80.2 (C-4), 102.0 ($J_{C1\text{-}H1}$162.6 C-1, β), 102.2 ($J_{C1\text{-}H1}$ 162.3 C-1' β), 103.4 ($J_{C1\text{-}H1}$ 159.4 C-1"β); ES HRMS Calcd. for $C_{28}H_{51}N_3O_{14}Na$ 676.3268. Found: 676.3269.

*6-(Fluoresceinylthioureido)hexyl (3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-(1→3)-(2-acetamido-2-deoxyβ-D-galactopyranosyl)-(1→4)-2-acetamido- 2-deoxy-β-D-glucopyranoside* (**63**).

The trisaccharide **2** (10 mg) was dissolved in 1 mL DMF:H_2O 1:1, containing sodium bicarbonate (20 mg). Fluorescein isothiocyanate (10mg) was added and the reaction was stirred for 1 hour. It was then quenched with ethylene diamine (20μL) and concentrated to a yellow film. This was taken up in water and purified by HPLC on C18 reverse phase silica using a 10-50% acetonitrile gradient. The fractions were collected and lyophized to give **63** (11 mg 75%) $[\alpha]_D$ – 12.7° (*c* 0.11, H_2O) ^1H (600 MHz, CD_3OD) 8.12 (s, 1 H, H-4fl), 7.76 (br d, 1H, H-6fl), 7.41 (d, 1 H, J 8.2 Hz, H-7fl), 6.67-6.52 (m, 4 H, H-2'fl, H-5'fl, H-7'fl), 4.56 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1"), 4.51 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1'), 4.35 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.07 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2'), 4.02 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4'), 3.88 (dd, J_{vic} 6.0, J_{gem} 9.7 Hz, OC$\underline{H}_2$CH$_2$) 3.80 (dd, 1 H, $J_{5,6a}$ 2.0, J_{gem} 12.0 Hz, H-6a), 3.78 (br t, 2 H, $J_{2,3ax}\approx J_{2,3eq}$ 3.4 Hz, H-2"), 3.76 (d, 1 H, J_{gem} 11.4 Hz, H-6a'), 3.75 (dd, $J_{2,3}$ 9.7 Hz, H-3'), 3.72 (dd, 1H, $J_{2,3}$ 10.3 Hz, H-2), 3.67 (dd, 1 H, $J_{5,6}$ 4.4 Hz, H-6b'), 3.62 (dd, $J_{5,6}$ 4.8 Hz, H-6b), 3.61-3.57 (m, 4 H, H-3, H-5', C$\underline{H}_2$NHCS), 3.52 (dd, $J_{3,4}\approx J_{4,5}$ 9.5 Hz, H-4), 3.48 (dd, 1 H, J_{vic} 2.2 Hz, CH$_2$C$\underline{H}_2$O), 3.46 (ddd, $J_{3eq,4}$ 4.4, $J_{3ax,4}\approx J_{4,5}$ 10.9 Hz, H-4"), 3.31 (ddd, 1 H, H-5), 3.27 (dq, 1 H, $J_{5,6}$ 6.2 Hz, H-5"), 2.11 (ddd, 1 H J_{gem} 13.5 Hz, H-3eq"), 1.97 (s, 3 H, C$\underline{H}_3$CONH), 1.96 (s, 3 H, C$\underline{H}_3$CONH), 1.69-1.63 (m, 2 H, C$\underline{H}_2$CH$_2$NHCS), 1.62-1.55 (m, 2 H, CH$_2$C$\underline{H}_2$CH$_2$O), 1.52 (ddd, 1 H, H3ax"), 1.48-1.37 (m, 4 H, CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$), 1.25 (d, 3 H, H-6"); ES HRMS Calcd. for $C_{49}H_{62}N_4O_{19}SNa$ 1065.3626. Found: 1065.3627.

*6-(Amino)hexyl (3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido- 2-deoxy-β-D-glucopyranoside* (**3**)

Trisaccharide **2** (5 mg, 0.0076 mmol) was dissolved in reaction buffer (2 mL) (20 mM HEPES, 20 mM $MnCl_2$, 0.2 % BSA pH 7), to which was added GDP-fucose (6 mg. 0.009 mmol) and 10 mU of α1,3-fucosyltransferase V (human recombinant, *Sodoptera fugiperda*, Calbiochem). The reaction was incubated for 48 h at 37 °C. The solution was then diluted with water and loaded onto a Sep-Pak C18 cartridge, washed with water (10 mL) and the product was

eluted with methanol. After concentration the product was dissolved in water and lyophilized to give a white solid (4.5 mg, 74%); ^{1}H (600 MHz, D_2O) (ring definitions A: GlcNAc, B: Fuc, C: GalNAc, D: Tyv) 5.10 (1 H, d, J_{1-2} 4.1 Hz, H-1B), 4.84 (1 H, q, J_{5-6} 6.7 Hz, H-5B), 4.70 (1 H, s, H-1D), 4.52 (1 H, d, J_{1-2} 8.6 Hz, H-1C) 4.45 (1 H, d, J_{1-2} 8.3 Hz, H-1A), 4.12 (1 H, d, J_{3-4} 3.4 Hz, 4C), 4.09 (1 H, dd, J_{2-3} 11.0 Hz, H-2C), 3.92-3.88 (6 H, m, H-2A, H-2D, H-4A, H-3B, H-6A, OCH_2CH_2), 3.84 (2 H, m, H-3C, H-4B), 3.80-3.70 (4 H, m, H-3A, H-6Ab, H-6C), 3.71 (1 H, dd, J_{2-3} 10.6 Hz, H-2B), 3.58 (1 H, dd, J_{5-6} 4.0, J_{5-6} 8.0 Hz, H-5C), 3.57 (1 H, dt, J_{gem} 10.1, J_{vic} 6.4 Hz, OCH_2CH_2), 3.55 (1 H, ddd, J_{3eq-4} 4.9, J_{3ax-4} 11.5, J_{4-5} 9.1 Hz, H4D), 3.52 (1 H, ddd, J_{4-5} 9.4, J_{5-6} 2.1, J_{5-6} 5.0 Hz, H-5A), 3.44 (1 H, dq, J_{5-6} 6.1 Hz, H-5D), 2.98 (2 H, t, J_{vic} 7.7 Hz, CH_2NH_2), 2.16 (1 H, ddd, J_{2-3eq} 3.7, J_{gem} 13.7 Hz, H-3eq), 2.04 (3 H, CH_3CO), 2.02 (3 H, s, CH_3CO), 1.66 (1 H, ddd, H-3Dax), 1.65 (2 H, m, $CH_2CH_2CH_2NH_2$), 1.55 (2 H, m, J 6.4 Hz, $CH_2CH_2CH_2O$), 1.40-1.30 (4 H, m, $CH_2CH_2CH_2CH_2$), 1.27 (3 H, d, H-6D), 1.25 (3 H, d, H-6B) ES HRMS for $C_{34}H_{62}N_3O_{18}$ 800.4028. Found: 800.4030.

Allyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranoside (**64**). Allyl *3,4,6-tri-O-benzyl-β-D-glucopyranoside* (**65**).

-3,4,6 Tri-O-benzyl-1,2-*O*-(*exo*-ethoxyethylidene)-α-D-glucopyranose (1.33 g, 2.6 mmol) was dissolved in allyl alcohol (25 mL) and cooled to 0 °C in an ice bath. $BF_3{\cdot}OEt_2$ (200μL, 1.6mmol) was added dropwise, the reaction was stirred at 0 °C for 30 min and then quenched with triethylamine. The solution was concentrated to a yellow oil and subject to column chromatography in toluene:ethylacetate (9:1) that gave **64** (646 mg, 43%) and **65** (670 mg, 53%) as white crystals that could be recrystallized from EtOAc-hexane mixtures. Data for **64** : m.p. 44-46 °C; $[\alpha]_D$ -8.3° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.15 (15 H, Ar), 5.84 (OCH_2CHCH_2), 5.24 ($OCH_2CHCHaHb$), 5.14 ($OCH_2CHCHaHb$), 5.02 (1 H, m, H-2), 4.78 (d, 2 H, J 11.1 Hz, OCH_2Ph), 4.66 (d, 1 H, J 11.4 Hz, OCH_2Ph), 4.58 (d, 1 H, J 10.4 Hz, OCH_2Ph), 4.54 (d, 1 H, J 12.2 Hz, OCH_2Ph), 4.40 (d, 1 H, H-1), 4.32 (OCH_2CHCH_2), 4.06 (OCH_2CHCH_2), 3.76-3.61 (4 H, H-3, H-4, H-6a, H-6b), 3.47 (ddd, 1 H, J_{4-5} 9.3, J_{5-6a} 2.3, J_{5-6b} 4.6 Hz, H-5), 1.96 (s, 3 H, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 138.9, 138.8, 138.6, 134.6, 129.1-128.3, 117.7, 100.6, 83.7, 78.7, 77.9, 75.9, 75.7, 74.2, 73.8, 70.3, 69.5; Anal. Calcd. For $C_{32}H_{36}O_7$; C, 72.16; H, 6.81; O, 21.03; Found; C, 71.99; H, 6.94

Data for **65**: m.p. 41-43 °C; $[\alpha]_D$ –3.0° (c 1.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.14 (15 H, Ar), 5.92 (OCH_2CHCH_2), 5.29 (OCH_2CHCH_2), 5.19 (OCH_2CHCH_2), 4.78 (d, 1 H, J 11.4 Hz, OCH_2Ph), 4.82 (d, 1 H, J 11.4 Hz, OCH_2Ph), 4.81 (d, 1 H, J 10.8 Hz, OCH_2Ph), 4.59 (d, 1 H, J 12.3 Hz, OCH_2Ph), 4.52 (d, 1 H, J 12.4 Hz, OCH_2Ph), 4.52 (d, 1 H, J 10.8 Hz, OCH_2Ph), 4.38

(OCH_2CHCH_2), 4.28 (ABX, 1 H, J_{1-2} 7.1 Hz, H-1), 4.10 (OCH_2CHCH_2), 3.72 (dd, 1 H, J_{gem} 10.8, J_{5-6a} 1.1 Hz, H-6a), 3.67 (dd, 1 H, J_{5-6b} 4.8 Hz, H-6b), 3.60-3.55 (3 H, m, H-2, H-3, H-4), 3.45 (ddd, 1 H, H-5), 1.96 (s, 3 H, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.3, 135.9, 135.8, 131.6, 126.1-125.3, 115.5, 99.4, 82.3, 75.3, 72.9, 72.8, 72.6, 72.4, 71.2, 67.9, 66.6; Anal. Calcd. For $C_{30}H_{34}O_6$; C, 73.45; H, 6.99; O, 19.57; Found; C, 73.09; H, 6.99.

Allyl 3,4,6 tri-O-*benzyl-β-D-mannopyranoside* (**66**).

Allyl glucopryanoside **65** (500 mg) was dissolved in freshly distilled dimethyl sulfoxide (8 mL) and acetic anhydride (4 mL) was added. The resulting solution was stirred for 18 h at room temperature and then concentrated under vacuo to give a yellow oil. This was dissolved in 1:1 dichloromethane:methanol (20 mL) and cooled to 0 °C. Sodium borohydride (100 mg) was added and the suspension was stirred for 30 min at 0 °C. The reaction was then diluted with dichloromethane and washed with 2% citric acid followed by brine. The organic layer was dried over sodium sulfate and concentrated to a yellow oil. Chromatography in toluene:EtOAc (4:1) on silica gel gave **66** (425 mg, 85%) as a white crystal; m.p. 52-53 °C; $[\alpha]_D$ –21.5° (*c* 1.2, $CHCl_3$); ^1H NMR (600 MHz, $CHCl_3$) δ 7.36-7.18 (15 H, Ar), 5.90 (OCH_2CHCH_2), 5.26 ($OCH_2CHCHaHb$), 5.19 ($OCH_2CHCHaHb$), 4.87 (d, 1 H, J 11.0 Hz, OCH_2Ph), 4.75 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.66 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.60 (d, 1 H, J 12.1 Hz, OCH_2Ph), 4.54 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.52 (d, 1 H, J 10.6 Hz, OCH_2Ph), 4.44 (d, 1 H, J_{1-2} 0.7 Hz, H-1), 4.40 (OCH_2CHCH_2), 4.11-4.08 (m, 2 H, H-2, OCH_2CHCH_2), 3.85 (dd, J_{4-5}≈J_{3-4}≈9.3 Hz, H-4), 3.76 (dd, 1 H, J_{gem} 10.8 J_{5-6a} 2.2 Hz, H-6a), 3.70 (dd, J_{5-6b} 5.3 Hz, H-6b), 3.55 (1 H, dd, J_{2-3} 3.1 Hz, H-3), 3.41 (ddd, 1 H, H-5), ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 137.9, 133.7, 128.5-127.6, 117.85, 98.6 (J^1_{c1-H1} 158.6), 81.6, 75.4, 75.1, 74.3, 73.5, 71.4, 69.9, 69.3, 68.4.

Allyl 2-O-*(3,4,6-tri*-O-*benzyl-β-D-mannopyranosyl)-3,4,6-tri*-O-*benzyl-β-D-mannopyranoside* (**67**).

Glycosyl acceptor **66** (1.0 g, 2.0 mmol) and silver zeolite (3.3 g) in dichloromethane (4 mL) was stirred for 20 min at room temperature. Freshly prepared 3,4,6 tri-*O*-benzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide (2.6 g, 5 mmol) was dissolved in dichloromethane (2 mL) and was added dropwise to the suspension containing the glycosyl acceptor. The reaction was stirred for 16 h, and then filtered through celite and concentrated to yellow syrup. This syrup was dissolved in tetrahydrofuran (30 mL) and cooled to –78 °C under argon. L-Selectride (1M THF, 8 mL) was then added dropwise and the reaction was stirred for 5 min, the dry ice bath was

removed and the reaction was allowed to warm to room temperature. The reaction mixture was quenched after 15 min with methanol (2 mL) and diluted with dichloromethane. Washing with a solution of hydrogen peroxide (5%) and sodium hydroxide (1M) followed by thiosulfate (5%) and brine solutions gave a clear colourless organic solution. This was dried over sodium sulfate and concentrated to a colourless oil. The oil was taken up in a 1:1 solution of toluene and EtOAc (2 mL) and passed through a plug of aluminium oxide (neutral, activated Brockman I, 3 cm) and the aluminium oxide was washed with the equivalent solution (90 mL). The combined filtrates were concentrated and subjected to column chromatography in toluene:EtOAc (8:1) to give **67** as a colourless syrup (1.47g, 78%), $[\alpha]_D$ –4.6° (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.39-7.12 (30 H, Ar), 5.85 ($OCH_2\underline{C}HCH_2$), 5.20 ($OCH_2CHCHa\underline{Hb}$), 5.13 ($OCH_2CH\underline{Ha}Hb$), 4.95 (s, 1 H, H-1'), 4.94 (d, 1 H, J 10.5 Hz, $OC\underline{H}_2Ph$), 4.91 (d, 1 H, J 11.6 Hz, $OC\underline{H}_2Ph$), 4.86 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.82 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.62 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.61 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 4.54 (d, 1 H, J 10.7 Hz, $OC\underline{H}_2Ph$), 4.54 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 4.50 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 4.46-4.43 (4 H, $OC\underline{H}_2Ph$), 4.41 (1 H, s, H-1), 4.38 ($OC\underline{H}_2CHCH_2$), 4.33 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2'), 4.00 ($OC\underline{H}_2CHCH_2$), 3.90 (dd, 1 H, $J_{3,4}\approx J_{4,5}$ 9.5 Hz, H-4'), 3.79-3.75 (3 H, H-6a, H-4, H-6a'), 3.72 (dd, 1 H, $J_{5,6b}$ 5.5, J_{gem} 10.7 Hz, H-6b), 3.64 (dd, 1 H, $J_{5,6b}$ 6.1, J_{gem} 10.5 Hz, H-6b'), 3.56 (dd, 1H, H-3'), 3.53 (dd, 1 H, $J_{3,4}$ 6.6 Hz, H-3), 3.49 (ddd, 1 H, $J_{5,6a}$ 1.5 Hz, H-5'), 3.40 (ddd, 1 H, $J_{5,6a}$ 1.5 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4-138.1, 133.8, 128.6-127.5, 117.1, 100.1 ($^1J_{C-H}$ 163 Hz, C-1'), 99.3 ($^1J_{C-H}$ 154 Hz, C-1), 81.5, 80.4, 80.3, 75.6, 75.2, 75.1, 75.1, 74.5. 74.1, 73.5, 73.4, 70.8, 70.7, 70.1, 70.1, 70.0, 69.5, 67.7; Anal. Calcd. For $C_{57}H_{62}O_{11}$; C, 74.16; H, 6.77; O, 19.07; Found; C, 73.87; H, 6.91.

*Allyl (3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl) (1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**68**) and *Allyl (3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl) (1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**69**).

3,4,6 Tri-O-benzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide (207 mg 0.22 mmol), glycosyl acceptor (**7**) (345 mg, 0.66 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (152 mg, 0.74 mmol) were dried together under vacuum for 1 hr in a pear shaped flask (25 mL). The contents were then dissolved in acetonitrile (2.0 mL), activated 3A molecular sieves (500 mg) and a stir bar were then added. The suspension was stirred for 30 min and an ambient temperature water bath was added to moderate the reaction temperature. Silver triflate (173 mg, 0.67 mmol) was added and the reaction was stirred in the dark for 1 h. The reaction was then diluted with dichloromethane and filtered through celite. Combined filtrates were washed with a solution of

sodium bicarbonate, dried over sodium sulphate and concentrated. The syrup was taken up in THF (10 mL) and cooled to –78 °C under argon. L-Selectride (3 mL, 1M in THF) was added dropwise and the cooling bath was removed. Once the reaction had reached ambient temperature methanol (2 mL) was added. The reaction was diluted with dichloromethane and washed with 5% hydrogen peroxide/1M NaOH followed by 5% thiosulfate/brine to give a clear solution, which after drying over sodium sulphate, and concentration, a colourless oil was obtained. Column chromatography in toluene:EtOAc (9:1) gave **68** (123 mg, 45%) as a colourless oil, **69** (28 mg, 11%) and 3,4-di-*O*-benzyl-1,6-anhydro-β-D-mannopyranose (46 mg, 20%, based on donor) **68**; $[\alpha]_D$ –62.2 °(*c* 2.2, $CHCl_3$); ^1H NMR (600 MHz, $CHCl_3$) δ 7.45-6.94 (m, 45 H, Ar), 5.84 ($OCH_2C\underline{H}CH_2$), 5.20 ($OCH_2CHCHa\underline{Hb}$), 5.16 (s, 1 H, H-1"), 5.13 ($OCH_2CHC\underline{Ha}Hb$), 5.10 (s, 1 H, H-1'), 4.97 (d, 1 H, J 11.5 Hz, $OC\underline{H}_2Ph$), 4.94 (d, 1 H, J 10.4 Hz, $OC\underline{H}_2Ph$), 4.92 (d, 1 H, J 10.5 Hz, $OC\underline{H}_2Ph$), 4.84 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.77 (d, 1 H, J 9.2 Hz, $OC\underline{H}_2Ph$), 4.69 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2'), 4.61 (d, 1 H, J 12.3 Hz, $OC\underline{H}_2Ph$), 4.60 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.52 (d, 1 H, J 11.0 Hz, $OC\underline{H}_2Ph$), 4.50-4.52 (m, 9 H, H-1, 8($OC\underline{H}_2Ph$), $OC\underline{H}_2CHCH_2$), 4.33 (d, 1 H, J 10.1 Hz, $OC\underline{H}_2Ph$), 4.29 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2"), 4.21 (d, 1 H, J 10.4 Hz, $OC\underline{H}_2Ph$), 4.02 (d, 1 H, J 11.8 Hz, $OC\underline{H}_2Ph$), 3.98 ($OC\underline{H}_2CHCH_2$), 3.89 (dd, $J_{3,4}\approx J_{4,5}$ 9.7 Hz, H-4'), 3.87 (dd, $J_{3,4}\approx J_{4,5}$ 9.6 Hz, H-4"), 3.80-3.48 (m, 11 H, H-5", H6a", H-6b", H-3', H-5', H-6a', H-6b', H-3, H-4, H-6a, H-6b), 3.46 (dd, 1H, $J_{3,4}$ 9.1 Hz, H-3"), 3.38 (ddd, 1 H, $J_{5,6a}$ 2.2, $J_{5,6b}$ 4.1, $J_{4,5}$ 9.0 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) 138.5, 138.4, 138.2, 138.1, 138.0, 138.0, 137.8, 133.5, 129.0-127.1, 117.3, 100.6 ($^1J_{C\text{-}H}$ 157 Hz), 100.1 ($^1J_{C\text{-}H}$ 161 Hz), 99.9 ($^1J_{C\text{-}H}$ 162 Hz), 80.3, 75.4, 75.4, 75.3, 75.2, 75.1, 75.1, 74.8, 74.5, 74.4, 73.5, 73.4, 73.4, 71.3, 70.3, 70.2, 70.1, 70.0, 69.8, 69.7, 67.6, 69.0, 67.4; ES Calcd. 1377.6 found 1377.6.

Trisaccharide **69** $[\alpha]_D$ –6.4° (*c* 0.92, $CHCl_3$) ^1H NMR (500 MHz, $CHCl_3$) δ 7.41-6.94 (m, 45 H, Ar), 5.82 ($OCH_2C\underline{H}CH_2$), 5.41 (d, 1 H, $J_{1\text{-}2}$ 3.7, H-1"), 5.17 ($OCH_2CHCHa\underline{Hb}$), 5.11 ($OCH_2CHC\underline{Ha}Hb$), 4.98 (s, 1 H, H-1'), 4.94 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.83 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.79 (d, 1 H, J 11.3 Hz, $OC\underline{H}_2Ph$), 4.77 (d, 1 H, J 10.1 Hz, $OC\underline{H}_2Ph$), 4.75 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.74 (d, 1 H, J 10.9 Hz, $OC\underline{H}_2Ph$), 4.62 (d, 1 H, J 11.7 Hz, $OC\underline{H}_2Ph$) 4.55-4.51 (m, 4 H, $OC\underline{H}_2Ph$), 4.45-4.34 (m, 9 H, 5 ($OC\underline{H}_2Ph$), H-2', H-2, H-1, $OC\underline{H}_2CHCH_2$), 4.23 (ddd, 1 H, $J_{5\text{-}6}\approx J_{5\text{-}6}$ 2.5, $J_{4\text{-}5}$ 9.9 Hz, H-5"), 4.21 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 4.17 (d, 1 H, J 10.7 Hz, $OC\underline{H}_2Ph$), 3.97 ($OC\underline{H}_2CHCH_2$), 3.87 (dd, $J_{3,4}\approx J_{4,5}$ 9.7 Hz, H-4'), 3.83 (dd, $J_{3\text{-}4}\approx J_{4\text{-}5}$ 9.3 Hz, H-3"), 3.73-3.58 (m, 9 H, H-2", H-6a", H-6b", H-3', H-6a', H-6b', H-4", H-4, H-6a, H-6b), 3.50 (dd, 1 , $J_{2\text{-}3}$ 3.6, $J_{3\text{-}4}$ 9.4 Hz, H-3), 3.46 (ddd, 1 H, $J_{5\text{-}6}$ 1.8, $J_{5\text{-}6}$ 5.5, $J_{4\text{-}5}$ 9.6 Hz, H-5'), 3.41 (1 H, ddd, $J_{5\text{-}6}$ 1.6, $J_{5\text{-}6}$ 6.7, $J_{4\text{-}5}$ 9.2 Hz, H-5), ^{13}C NMR (125 MHz, $CDCl_3$) 139.2, 138.6, 138.4, 138.4, 138.1, 138.0, 138.0, 137.2, 133.8, 128.5-127.3, 117.3, 100.3 ($^1J_{C\text{-}H}$ 156 Hz), 99.5 ($^1J_{C\text{-}H}$ 161 Hz), 99.9

($^1J_{C-H}$ 172 Hz), 88.8, 83.0, 80.4, 75.8, 75.4, 75.3, 75.1, 75.1, 75.0, 74.8, 74.6, 74.4, 73.4, 73.3, 72.4, 71.0, 70.6, 70.6, 70.2, 70.3, 69.7, 69.8; ES Calcd. 1377.6 found 1377.6.

3,4,6 Tri-O-4-chlorobenzyl-1,2-O-(exo-ethoxyethylidene)-α-D-glucopyranose (**70**).

3,4,6 Tri-*O*-acetyl-1,2-*O*-(*exo*-ethoxyethylidene)-α-D-glucopyranose (6.6 g, 17 mmol) was dissolved in methanol (50 mL) and sodium metal (~20 mg) was added. The solution was stirred at room temperature for 1 h. The solution was concentrated to a colourless oil and azeotroped twice from toluene (20 mL). The resulting oil was taken up in DMF (100 mL) and sodium hydride (1.34 g 55.8 mmol) was added followed by 4-chorobenzyl chloride (8.7 g, 54 mmol). The reaction was stirred overnight and quenched with the addition of triethylamine (10 mL) followed 30 min later with methanol (10 mL). The suspension was concentrated to a yellow oil, diluted with dichloromethane and washed with water, dried and concentrated. Column chromatography of the resulting yellow syrup in toluene:EtOAc (6:1), 1% triethylamine gave a colourless syrup (10.5 g, 96%); ^{1}H NMR (600 MHz, $CHCl_3$) δ 7.29-7.06 (15 H, Ar), 5.73 (d, 1 H, J_{1-2} 5.3 Hz, H-1), 4.67 (d, 1 H, J 12.1 Hz, OC$\underline{H}_2$Ph), 4.54 (d, 1 H, J 11.7 Hz, OC$\underline{H}_2$Ph), 4.52 (d, 1 H, J 12.3 Hz, OC$\underline{H}_2$Ph), 4.52 (d, 1 H, J 11.9 Hz, OC$\underline{H}_2$Ph), 4.43 (d, 1 H, J 12.3 Hz, OC$\underline{H}_2$Ph), 4.37 (dd, 1 H, J_{2-3} 3.5 Hz, H-2), 4.35 (d, 1 H, J 11.4 Hz, OC$\underline{H}_2$Ph), 3.79 (dd, 1 H, J_{3-4} 5.0 Hz, H-3), 3.74 (ddd, 1 H, $J_{4-5}\approx J_{5-6b}$ 2.7, J_{5-6a} 9.7 Hz, H-5), 3.64 (dd, 1 H, J_{6a-6b} 5.0 Hz, H-6a), 3.62-3.61 (2 H, H-6b, H-4), 3.52 (2 H, ABX_3, OC$\underline{H}_2$CH$_3$), 1.64 (s, 3 H, O_2CC$\underline{H}_3$), 1.18 (t, J_{vic} 7.0 Hz, OCH$_2$C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 137.2, 137.0, 136.8, 129.9-129.2, 121.7, 98.5, 80.0, 77.5, 76.6, 73.4, 73.0, 71.8, 71.1, 69.8, 59.5, 22.6, 16.0; Anal. Calcd. For $C_{31}H_{33}O_7Cl_3$; C, 59.67; H, 5.33; O 17.65; Cl, 17.05; Found; C, 59.74, H, 5.23.

2-O-Acetyl-1,5-anhydro-3,4,6-tri-O-4-chlorobenzyl-D-arabino-hex-1-enitol (2-Acetoxy-3,4,6 Tri-O-4-chlorobenzyl-D-glucal)(**71**).

Othoester **70** (560 mg) was dissolved in bromobenzene (50 ml) the solution was brought to reflux and pyridine (0.5 mL) was added. Reaction was monitored by TLC (8:1 toluene/EtOAc) and after 2 hr no starting material remained. The reaction was then concentrated and purified by column chromatography toluene:EtOAc (9:1) to give a colourless oil (481 mg, 93%); $[\alpha]_D$ 12.4° (*c* 1.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CHCl_3$) δ 7.29-7.12 (8 H, Ar), 6.58 (s, 1 H, H-1), 4.65 (d, 1 H, J 11.7 Hz, OC$\underline{H}_2$Ph), 4.55 (d, 1 H, J 11.7 Hz, OC$\underline{H}_2$Ph), 4.53-4.44 (m, 4 H, OC$\underline{H}_2$Ph), 4.40 (d, 1 H, J_{3-4} 5.3 Hz, H-3), 4.16 (ddd, 1 H, J_{4-5} 7.42, J_{5-6a} 5.0, J_{5-6b} 9.4 Hz, H-5), 3.90 (dd, 1 H, H-4), 3.77 (dd, 1 H, J_{gem} 10.8 Hz, H-6a), 3.68 (dd, 1 H, H-6b), 2.05 (s, 3 H, COC$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 139.1, 137.2, 137.0, 136.9, 134.4, 134.3, 134.2, 130.3, 129.7-129.3, 76.1,

74.6, 73.4, 73.0, 71.8, 68.6, 21.4; Anal. Calcd. For $C_{29}H_{27}O_6Cl_3$; C, 60.27; H, 4.71; O 16.61; Cl, 18.41; Found; C, 60.03, H, 4.68.

3,4,6 Tri-O-*4-chlorobenzyl-α-D*-arabino-*hexopyranos-2-ulosyl bromide* (**72**).

Glycal **71** (500 mg, 0.868 mmol) was dissolved in dichloromethane (6.5 mL) and freshly dried 3A molecular sieves were added. The solution was stirred under argon for 20 min and then the reaction was cooled to 0 °C. Anhydrous ethanol (60 μL, 1.02) was added followed by *N*-bromosuccinimide (183 mg, 0.102 mmol) and stirring was continued at 0 °C. When the solution turned pale yellow, approximately 1-2 minutes after *N*-bromosuccinimide addition, it was diluted with dichloromethane and filtered into an ice cold solution of thiosulfate. The organic layer was separated and diluted with EtOAc to a 1:1 mixture. This solution was filtered through a plug of silica gel (3 cm) and concentrated to a pale yellow syrup (493 mg, 92%) sufficiently pure for glycosylations. ^{1}H NMR (600 MHz, $CHCl_3$) δ 7.30-7.04 (15 H, Ar), 6.34 (s, 1 H, H-1), 4.94 (d, 1 H, J 11.4 Hz, OC$\underline{H}_2$Ph), 4.84 (d, 1 H, $J_{2\text{-}3}$ 9.7 Hz, H-3), 4.76 (d, 1 H, J 11.2 Hz, OC$\underline{H}_2$Ph), 4.55 (d, 1 H, J 11.4 Hz, OC$\underline{H}_2$Ph), 4.51 (d, 1 H, J 12.5 Hz, OC$\underline{H}_2$Ph), 4.49 (d, 1 H, J 11.5 Hz, OC$\underline{H}_2$Ph), 4.41 (d, 1 H, J 12.1 Hz, OC$\underline{H}_2$Ph), 4.20 (ddd, 1 H, $J_{4\text{-}5}$ 9.9, $J_{5\text{-}6a}$ 3.3, $J_{5\text{-}6b}$ 1.6 Hz, H-5), 3.95 (dd, 1 H, H-4), 3.80 (dd, J_{gem} 11.0 Hz, H-6a), 3.67 (dd, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) 195.0, 136.6, 136.2, 134.8, 134.6, 134.5, 131.6, 130.3-129.2, 86.2 ($J^1_{c1\text{-}H1}$ 189 Hz), 82.1, 77.6, 76.2, 75.2, 74.0, 73.5, 73.4, 67.9 ; ES HRMS Calcd. for $C_{27}H_{24}BrCl_3O_5Na$ 634.9770 found 634.9778.

Allyl (3,4,6-tri-O-p-*chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri*-O-*benzyl-β-D-mannopyranoside* (**73**) and *Allyl (3,4,6-tri*-O-p-*chlorobenzyl-α-D-glucoopyranosyl)(1→2)(3,4,6-tri*-O-*benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri*-O-*benzyl-β-D-mannopyranoside*(**74**)

Glycosyl donor (**72**) (820 mg 1.3 mmol), glycosyl acceptor (**67**) (380 mg, 0.41 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (330 mg, 1.6 mmol) were dried together under vacuum for 1 hr in a pear shaped flask (25 ml). The contents were then dissolved in acetonitrile (1.5 mL), activated 3A molecular sieves (500 mg) and a stir bar were added. The suspension was stirred for 30 min and an ambient temperature water bath was added to moderate the temperature of the reaction. Silver triflate (355 mg, 1.4 mmol) was then added and the reaction was stirred in the dark for 1 hr. The reaction was diluted with dichloromethane and filtered through celite. Combined filtrates were washed with a solution of sodium bicarbonate, dried over sodium sulphate and concentrated. The syrup was then taken up in THF (10 ml) and cooled to –78 °C

under argon. L-Selectride (5 mL, 1M in THF) was added dropwise and the cooling bath removed. Once the reaction had reached ambient temperature methanol (2 mL) was added. The reaction was diluted with dichloromethane and washed with 5% hydrogen peroxide/1M NaOH followed by 5% thiosulfate/brine to give a clear solution, which after drying over sodium sulphate, and concentration gave a colourless oil. The combined filtrates were concentrated and column chromatography in toluene:EtOAc (9:1) gave **73** (390 mg, 65%) and the α gluco epimer **74** (96 mg, 16%); $[\alpha]_D$ –64° (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CHCl_3$) δ 7.45-6.87 (42 H, Ar), 5.86 ($OCH_2C\underline{H}CH_2$), 5.20 ($OCH_2CHCHa\underline{Hb}$), 5.16 (s, 1 H, H-1"), 5.14 ($OCH_2CHC\underline{Ha}Hb$), 5.11 (s, 1 H, H-1'), 4.92 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.92 (d, 1 H, J 10.5 Hz, $OC\underline{H}_2Ph$), 4.92 (d, 1 H, J 10.2 Hz, $OC\underline{H}_2Ph$), 4.72 (d, 1 H, J 11.7 Hz, $OC\underline{H}_2Ph$), 4.71 (d, 1 H, J 9.2 Hz, $OC\underline{H}_2Ph$), 4.68 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2'), 4.63 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.62 (d, 1 H, $J_{2,3}$ 2.5 Hz, H-2), 4.51 (d, 1 H, J 10.3 Hz, $OC\underline{H}_2Ph$), 4.50 (d, 1 H, J 11.5 Hz, $OC\underline{H}_2Ph$), 4.44 (s, 1 H, H-1), 4.44-4.38 (6H, 5 $OC\underline{H}_2Ph$, $OC\underline{H}_2CHCH_2$), 4.29 (d, 1 H, J 9.7 Hz, $OC\underline{H}_2Ph$), 4.28 (d, 1 H, J 12.6 Hz, $OC\underline{H}_2Ph$), 4.25 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2"), 4.17 (d, 1 H, J 10.3 Hz, $OC\underline{H}_2Ph$), 4.04 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 3.99 ($OC\underline{H}_2CHCH_2$), 3.91 (dd, $J_{3,4}\approx J_{4,5}$ 9.5 Hz, H-4'), 3.82 (d, 1 H, J 12.0 Hz, $OC\underline{H}_2Ph$), 3.80 (dd, $J_{3,4}\approx J_{4,5}$ 9.0 Hz, H-4"), 3.77 (dd, J_{gem} 11.1 Hz, H-6a'), 3.72 (dd, $J_{5,6}$ 5.7 Hz, H-6b'), 3.27-3.67 (ABX, 2 H, H-6a, H-6b), 3.65-3.62 (3 H, m, H-4, H-6a", H-6b") 3.60 (dd, 1 H, $J_{3,4}$ 7.9 Hz, H-3'), 3.58 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 7.5 Hz, H-3), 3.50 (ddd, 1 H, $J_{5,6a}$ 1.1 Hz, H-5'), 3.46 (ddd, 1 H, $J_{5,6a}$ 2.8, $J_{5,6b}$ 4.3 Hz, H-5"), 3.39 (dd, 1 H, H-3"), 3.37 (ddd, 1 H, $J_{5,6a}$ 2.2, $J_{5,6b}$ 4.0, $J_{4,5}$ 8.7 Hz, H-5) ^{13}C NMR (125 MHz, $CDCl_3$) 139.1, 139.0, 138.8, 138.8, 138.6, 137.7, 137.4, 134.3, 134.0, 133.9, 133.6, 130.0-128.2, 118.2, 101.4 ($^1J_{C\text{-}H}$ 156 Hz), 100.7 ($^1J_{C\text{-}H}$ 164 Hz), 100.6 ($^1J_{C\text{-}H}$ 161 Hz), 83.8, 81.3, 81.1, 76.1, 76.1, 75.9, 75.8, 75.7, 75.4, 75.0, 74.9, 74.2, 74.1, 73.3, 73.1, 71.1, 70.8, 70.6, 70.5, 70.4, 70.1, 69.8, 69.4, 67.9; Anal. Calcd. For $C_{84}H_{87}Cl_3O_{16}$; C, 69.15; H, 6.01; O, 17.55; Cl, 7.29; Found; C, 69.26; H, 6.13;

Trisaccharide **74**; $[\alpha]_D$ –7.2 °(c 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CHCl_3$) δ 7.42-6.82 (42 H, Ar), 5.83 ($OCH_2C\underline{H}CH_2$), 5.41 (d, $J_{1\text{-}2}$ 3.75 Hz, H-1"), 5.19 ($OCH_2CHCHa\underline{Hb}$), 5.14 ($OCH_2CHC\underline{Ha}Hb$), 5.01 (s, 1 H, H-1'), 4.94 (d, 1 H, J 11.0 Hz, $OC\underline{H}_2Ph$), 4.84 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.79 (d, 1 H, J 11.6 Hz, $OC\underline{H}_2Ph$), 4.75 (d, 1 H, J 10.4 Hz, $OC\underline{H}_2Ph$), 4.71 (d, 1 H, J 11.6 Hz, $OC\underline{H}_2Ph$), 4.65 (d, 1 H, J 11.6 Hz, $OC\underline{H}_2Ph$), 4.62 (d, 1 H, J 10.3 Hz, $OC\underline{H}_2Ph$), 4.55 (s, 2 H, $OC\underline{H}_2Ph$), 4.54 (d, 1 H, J 10.7 Hz, $OC\underline{H}_2Ph$), 4.47 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 4.46 (d, 1 H, J 12.2 Hz, $OC\underline{H}_2Ph$), 4.42-4.35(m, 7 H, H-1, H-2', $OC\underline{H}_2CHCH_2$, 4 ($OC\underline{H}_2Ph$)), 4.31 (d, 1 H, J 11.2 Hz, $OC\underline{H}_2Ph$), 4.22 (ddd, 1 H, $J_{4\text{-}5}$ 9.9, $J_{5\text{-}6}\approx J_{5\text{-}6}$ 2.5 Hz, H-5"), 4.18 (d, 1 H, J 10.6 Hz, $OC\underline{H}_2Ph$), 4.12 (d, 1 H, J 12.5 Hz, $OC\underline{H}_2Ph$), 3.99 ($OC\underline{H}_2CHCH_2$), 3.78 (dd, $J_{2\text{-}3}\approx J_{3\text{-}4}$ 9.3 Hz, H-4'), 3.75-3.55 (m, 10 H, H-2", H-4". H-6a", H-6b", H-3', H-6a', H-6b', H-4, H-6a, H-6b), 3.53

(dd, 1 H, J_{3-4} 9.2 Hz, H-3), 3.47 (1 H, ddd, J_{5-6} 1.9, J_{5-6} 5.3 Hz, H-5'), 3.42 (ddd, J_{5-6} 1.9, J_{5-6} 6.4, J_{4-5} 9.7 Hz, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) 138.4, 138.3, 138.2, 137.9, 137.9, 137.6, 137.2, 136.9, 136.5, 133.7, 133.2, 133.1, 132.9, 129.0-127.5, 118.2, 100.4 ($^1J_{C-H}$ 154 Hz), 99.3 ($^1J_{C-H}$ 171 Hz), 99.1 ($^1J_{C-H}$ 160.7 Hz), 83.8, 83.0, 80.5, 77.2, 75.2, 75.6, 75.3, 75.3, 75.1, 75.0, 74.5, 74.6, 74.6, 74.1, 73.4. 73.3, 72.7. 72.5, 72.4, 70.9, 70.4, 70.1, 70.0, 69.8, 69.7, 68.8.

*Allyl (3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside* (**75**).

Glycosyl donor (**72**) (1.0 g, 1.6 mmol), glycosyl acceptor (**73**) (640 mg, 0.44 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (400 mg, 1.9 mmol) were dried together under vacuum for 1 hr in a pear shaped flask (25ml). The contents were then dissolved in a pivaloylnitrile: dichloromethane solution (2:1, 3 mL), activated 4 A molecular sieves (300mg) and a stir bar were added to the flask. The suspension was stirred for 30 min at room temperature under argon and the temperature was cooled to –40 °C by an ethanol bath. Silver triflate was added and the reaction was allowed to warm to 0 °C over 3 hr then warmed to room temperature for an additional 15 minutes. The reaction was diluted with dichloromethane and filtered through celite. Combined filtrates were washed with a solution of sodium bicarbonate, dried over sodium sulphate and concentrated. The syrup was then taken up in THF (10 mL) and cooled to –78 °C under argon. L-Selectride (3 mL, 1M in THF) was added dropwise and the cooling bath was removed. Once the reaction had reached ambient temperature methanol (2 mL) was added. The reaction was diluted with dichloromethane and washed with 5% hydrogen peroxide/1M NaOH followed by 5% thiosulfate/brine to give a clear solution. After drying over sodium sulfate and concentration a colourless oil was obtained. Column chromatography in toluene:EtOAc (9:1) gave **75** (480 mg, 55%), and the α gluco epimer (103mg, 12%) $[\alpha]_D$ -68° (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz $CDCl_3$) δ 7.45-6.88 (54 H, Ar), 5.82 ($OCH_2C\underline{H}CH_2$), 5.48 (s, 1 H, H-1"), 5.19 ($OCH_2CHCHa\underline{Hb}$), 5.16 (s, 1 H, H-1'''), 5.13 ($OCH_2CHC\underline{Ha}Hb$), 5.11 (s, 1 H, H-1'), 4.88 (d, 2 H, J 10.6 Hz, 2($OC\underline{H}_2Ph$)), 4.84 (d, 1 H, J_{2-3} 3.3 Hz, H-2'), 4.80 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.78 (d, 1 H, J 10.3 Hz, $OC\underline{H}_2Ph$), 4.72 (d, 2 H, J 11.2 Hz, 2($OC\underline{H}_2Ph$)), 4.57 (d, 1 H, J 12.5 Hz, $OC\underline{H}_2Ph$), 4.56 (d, 1 H, J 12.3 Hz, $OC\underline{H}_2Ph$), 4.52 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 4.52 (d, 1 H, J_{2-3} 3.1 Hz, H-2), 4.47 (d, 1 H, J 11.2 Hz, $OC\underline{H}_2Ph$), 4.47 (d, 1 H, J_{2-3} 3.3 Hz, H-2"), 4.45 (d, 1 H, J 12.8 Hz, $OC\underline{H}_2Ph$), 4.41 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.40 (s, 1 H, H-1), 4.40 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.38 (d, 2 H, J 12.0 Hz, 2($OC\underline{H}_2Ph$)), 4.38 ($OC\underline{H}_2CHCH_2$), 4.36 (d, 1 H, J 13.5 Hz, $OC\underline{H}_2Ph$), 4.32 (d, 1 H, H-2'''), 4.31 (d, 1 H, J 12.4 Hz, $OC\underline{H}_2Ph$), 4.30 (d, 2 H, J 10.0 Hz,

OCH_2Ph), 4.30 (d, 1 H, J 9.6 Hz, OCH_2Ph), 4.26 (d, 1 H, J 12.1 Hz, OCH_2Ph), 4.08 (d, 1 H, J 12.1 Hz, OCH_2Ph), 3.96 (OCH_2CHCH_2), 3.92 (dd, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4"), 3.90 (d, 1 H, J 12.1 Hz, OCH_2Ph), 3.83 (dd, 1 H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, H-4'"), 3.75 (d, 1 H, J 11.1 Hz, OCH_2Ph), 3.76-3.60 (12 H, H-4, H-6a, H-6b, H-3', H-4', H-6a', H-6b', H-6a", H-6b", H-6a'", H-6b'", OCH_2Ph), 3.58 (ddd, 1 H, H-5"), 3.56 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-3), 3.50 (ddd, 1 H, H-5'"), 3.49 (1 H, ddd, H-5'), 3.46 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3"), 3.44 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 9.2 Hz, H-3'"), 3.33 (ddd, 1 H, $J_{4,5}$ 9.5 $J_{5,6a}$ 1.7, $J_{5,6b}$ 4.0 Hz, H-5) ^{13}C NMR (125 MHz, $CDCl_3$), 138.9, 138.8, 138.7, 138.6, 138.5, 138.5, 137.9, 137.6, 137.5, 137.4, 137.4, 137.2, 134.3, 134.9, 134.9, 133.7, 133.6, 129.8-128.4, 118.1, 101.8 ($^1J_{C\text{-}H}$ 163 Hz, C1"), 101.4 ($^1J_{C\text{-}H}$ 162 Hz, C1'"), 100.9 ($^1J_{C\text{-}H}$ 161 Hz, C1'), 100.4 ($^1J_{C\text{-}H}$ 155 Hz, C1"), 83.7, 82.3, 81.4, 81.0, 76.2, 76.1, 75.9, 75.9, 75.8, 75.3, 75.1, 75.0, 74.9, 74.9, 74.2, 74.2, 73.5, 73.3, 73.1, 71.1, 71.2, 71.0, 71.0, 70.7, 70.5, 70.3, 70.2, 69.9, 69.8, 69.5, 69.3, 68.1; EMS Calcd. 1995 Found (M+Na) 2018 isotope intensities correct for Cl_6.

*Allyl (3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl) (1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**77**).

Glycosyl donor (**71**) (240 mg, 0.39 mmol), glycosyl acceptor (**75**) (190 mg, 0.095 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (120 mg, 0.59 mmol) were dried together under vacuum for 1 hr in a pear shaped flask (25ml). The contents of the flask were then dissolved in a pivaloylnitrile (800 µL)-dichloromethane (400 µL) solution, activated 4A molecular sieves (200 mg) and a stir bar were then added. The suspension was stirred for 30 min at room temperature under argon and then the temperature was reduced with a –40 °C bath. Silver triflate was added and the reaction was stirred in the dark. The flask was slowly warmed over 4 h to room temperature. The reaction was then diluted with dichloromethane and filtered through celite. Combined filtrates were washed with a solution of sodium bicarbonate, dried over sodium sulphate and concentrated. The syrup was then taken up in THF (10 mL) and cooled to –78 °C under argon. L-Selectride (2 mL, 1M in THF) was added dropwise and the cooling bath removed. Once the reaction had reached ambient temperature methanol (1 mL) was added. The reaction was diluted with dichloromethane and washed with 5% hydrogen peroxide/1M NaOH followed by 5% thiosulfate/brine to give a clear solution. After drying over sodium sulphate, concentration gave a colourless oil. Column chromatography in toluene:EtOAc (9:1) gave the pentasaccharide (123 mg, 51%), $[\alpha]_D$ -48° (*c* 0.94, $CHCl_3$); ^1H NMR (600 Hz $CDCl_3$) δ 7.45-6.65 (66 H, Ar), 5.83 (OCH_2CHCH_2), 5.53 (s, 1 H, H-1"), 5.53 (s, 1 H, H-1'"), 5.18

($OCH_2CHCHaHb$), 5.12 ($OCH_2CHCHaHb$), 5.13 (s, 1 H, H-1''''), 5.11 (s, 1 H, H-1'), 4.90 (d, 1 H, J_{gem} 11.3 Hz, OCH_2Ph), 4.88 (d, 1 H, J_{gem} 10.8 Hz, OCH_2Ph), 4.87 (d, 1 H, J_{gem} 9.7 Hz, OCH_2Ph), 4.85 (d, 1 H, J_{gem} 9.7 Hz, OCH_2Ph), 4.80 (d, 1 H, J_{2-3} 3.1 Hz, H-2'), 4.72 (d, 1 H, J_{gem} 11.1 Hz, OCH_2Ph), 4.70 (d, 1 H, J_{gem} 11.2 Hz, OCH_2Ph), 4.63 (d, J_{2-3} 3.1 Hz, H-2''), 4.56 (d, 2 H, J 11.7 Hz, 2(OCH_2Ph)), 4.54 (d, 1 H, J_{gem} 12.3 Hz, OCH_2Ph), 4.52 (d, 1 H, J_{2-3} 3.7 Hz, H-2), 4.52 (d, 1 H, J_{gem} 9.3 Hz, OCH_2Ph), 4.50 (d, 1 H, J_{gem} 9.7 Hz, OCH_2Ph), 4.48 (d, 1 H, J_{2-3} 3.3 Hz, H-2'''), 4.45-4.34 (m, 13 H, 11(OCH_2Ph), H-1, OCH_2CHCH_2), 4.31 (d, 1 H, J_{gem} 9.7 Hz, OCH_2Ph), 4.31 (d, 1 H, J_{2-3} 3.3 Hz, H-2''''), 4.25 (d, 1 H, J_{gem} 12.3 Hz, OCH_2Ph), 4.23 (d, 1 H, J_{gem} 12.1 Hz, OCH_2Ph), 4.15 (d, 1 H, J_{gem} 11.1 Hz, OCH_2Ph), 4.12 (d, 1 H, J_{gem} 11.7 Hz, OCH_2Ph), 3.96 (OCH_2CHCH_2), 3.93 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.5 Hz, H-4'''), 3.89 (d, 1 H, J_{gem} 11.9 Hz, OCH_2Ph), 3.84 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.3 Hz, H-4), 3.79 (d, 2 H, J_{gem} 12.3 Hz, OCH_2Ph), 4.79 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.5 Hz, H-4''), 3.78- 3.54 (m, 17 H, H-5'', H-6a'', H-6b'', H-5''', H-6a''',H-6b''', H-4'''', H-5'''', H-6a'''', H-6b'''', H-4', H-6a', H-6b', H-3', H-3, H-4, H-6, H-6, OCH_2Ph), 3.50-3.44 (m , 3 H, H-3'', H-3''', H-5'), 3.42 (dd, J_{3-4} 9.2 Hz, H-3''''), 3.33 (ddd, J_{4-5} 9.7, J_{5-6} 2.0, J_{5-6} 4.2 Hz, H-5); ^{13}C NMR (125 Hz, $CDCl_3$), 138.2-135.2, 133.5-133.0, 129.8-127.5, 117.3, 101.1 (J^1_{C-H} 162.8 Hz, C1''),100.7 (J^1_{C-H} 163.3 Hz, C1''''), 100.4 (J^1_{C-H} 162.8 Hz, C1'''), 100.3 (J^1_{C-H} 154.2 Hz, C1), 99.9 (J^1_{C-H} 160. Hz, C1'), 82.9, 81.6, 81.6, 80.4, 80.3, 75.6, 75.6, 75.5, 75.3, 75.2, 75.1, 75.1, 74.7, 74.3, 74.3, 74.0, 73.5, 73.4, 72.8, 72.7, 72.7, 72.7, 70.5, 70.3, 70.1, 70.0, 69.7, 69.7, 69.4, 69.4, 69.4, 69.3, 68.9, 68.9, 68.7, 68.7, 67.4, 67.4; EMS Calcd. (M+Na) 2553.6 found 2553.7 correct isotope intensities for Cl_9.

*Allyl (3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl) (1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**78**).

Procedure analogous to preparation of **77**. Glycosyl donor **71** (143 mg, 0.23 mmol), glycosyl acceptor (**77**) (153 mg, 0.060 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (106 mg, 0.52 mmol), pivaloylnitrile (500 µL), dichloromethane (250 µL), activated 4A molecular sieves (148 mg). Column chromatography in toluene:EtOAc (9:1) gave the hexasaccharide **78** (89 mg, 48%), $[\alpha]_D$ -61° (*c* 0.4, $CHCl_3$); ^1H NMR (600 Hz $CDCl_3$) δ 7.42-6.59 (66 H, Ar), 5.80 (OCH_2CHCH_2), 5.56 (s, 1 H, H-1'''), 5.53 (s, 1 H, H-1''), 5.43 (s, 1 H, H-1''''), 5.17 ($OCH_2CHCHaHb$), 5.11 (s, 1 H, H-1''''''), 5.10 ($OCH_2CHCHaHb$), 5.08 (s, 1 H, H-1'), 4.89 (d, 1 H, J_{gem} 10.1 Hz, OCH_2Ph), 4.87 (d, 1 H, J_{gem} 10.6 Hz, OCH_2Ph), 4.85 (d, 1 H, J_{gem} 11.2 Hz, OCH_2Ph), 4.80 (d, 1 H, J_{gem} 10.4

Hz, OC$\underline{H}_2$Ph), 4.79 (d, 1 H, $J_{2\text{-}3}$ 3.1 Hz, H-2'), 4.69 (d, 2 H, J_{gem} 11.1 Hz, OC$\underline{H}_2$Ph), 4.63 (d, 1 H, J_{gem} 11.0 Hz, OC$\underline{H}_2$Ph), 4.61 (d, $J_{2\text{-}3}$ 3.3 Hz, H-2''), 4.60 (d, $J_{2\text{-}3}$ 3.8 Hz, H-2'''), 4.54 (d, 1 H, J 12.4 Hz, OC$\underline{H}_2$Ph), 4.54 (d, 1 H, J_{gem} 11.1 Hz, OC$\underline{H}_2$Ph), 4.50 (d, 1 H, J_{gem} 12.3 Hz, OC$\underline{H}_2$Ph), 4.50 (d, 1 H, $J_{2\text{-}3}$ 3.1 Hz, H-2), 4.49-4.20 (m, 22 H, H-2'''', H-1, H-2''''', 18(OC$\underline{H}_2$Ph)), 4.22 (d, 1 H, J_{gem} 10.6 Hz, OC$\underline{H}_2$Ph), 4.18 (d, 1 H, J_{gem} 12.1 Hz, OC$\underline{H}_2$Ph), 4.18 (d, 1 H, J_{gem} 12.5 Hz, OC$\underline{H}_2$Ph), 4.09 (d, 1 H, J_{gem} 11.9 Hz, OC$\underline{H}_2$Ph), 4.08 (d, 1 H, J_{gem} 11.0 Hz, OC$\underline{H}_2$Ph), 3.93 (OC$\underline{H}_2$CHCH$_2$), 3.91 (dd, 1 H, $J_{3\text{-}4}$≈$J_{4\text{-}5}$ 9.7 Hz, H-4''''), 3.87 (dd, 1 H, $J_{3\text{-}4}$≈$J_{4\text{-}5}$ 9.3 Hz, H-4'''''), 3.84 (d, 1 H, J_{gem} 11.7 Hz, OC$\underline{H}_2$Ph), 3.83-3.53 (m, 23 H, H-3, H-4, H-6a, H-6b, H-3', H-4', H-5', H-6a', H-6b', H-4'', H-5'', H-6a'', H-6b'', H-4''', H-6a''', H-6b''', H-6a'''', H-6b'''', H-6a''''', H-6b''''', 3(OC$\underline{H}_2$Ph)), 3.50 (ddd, 1 H, $J_{4\text{-}5}$ 8.1, $J_{5\text{-}6}$ 4.1, $J_{5\text{-}6}$ 1.8 Hz, H-5'''''), 3.45-3.37 (m, 4 H, H-3''''', H-3'', H-3''', H-3'''', H-3''''', H-5'''), 3.31 (ddd, $J_{4\text{-}5}$ 9.7, $J_{5\text{-}6}$ 2.0, $J_{5\text{-}6}$ 4.2 Hz, H-5); ^{13}C NMR (125 Hz, $CDCl_3$), 138.1-135.9, 133.6-133.0, 129.8-127.5, 117.3, 101.2 ($J^1_{C\text{-}H}$ 163.0 Hz, C1''''), 100.7 ($J^1_{C\text{-}H}$ 163.6 Hz, C1'''''), 100.5 ($J^1_{C\text{-}H}$ 163.4 Hz, C1''), 100.4 ($J^1_{C\text{-}H}$ 163.5 Hz, C1'''), 100.2 ($J^1_{C\text{-}H}$ 155.1 Hz, C1), 100.0 ($J^1_{C\text{-}H}$ 159.5 Hz, C1'), 83.1, 83.0, 81.8, 81.8, 81.7, 80.6, 80.4, 75.8, 75.7, 75.6, 75.5, 75.4, 75.3, 75.3, 75.2, 75.2, 75.0, 74.8, 74.4, 74.4, 74.3, 74.3, 73.6, 72.9, 72.9, 72.8, 72.7, 70.7, 70.5, 70.4, 70.3, 70.1, 69.8, 69.7, 69.5, 69.4, 69.4, 69.3, 69.3, 69.2, 69.1, 68.9, 68.8, 67.5; EMS Calcd. for $C_{165}H_{162}Cl_{12}O_{31}Na$ 3081.7 found 3081.7 correct isotope pattern for Cl_{12}.

3-(2-Aminoethylthio)-propyl (3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**79**).

Allyl disaccharide (100 mg) (**67**) was added to a quartz vessel and dissolved in a minimum amount of dichloromethane (500 μL). Methanol (5 mL) was then added followed by 2-aminoethanethiol hydrochloride (1.0 g). The reaction was stirred until dissolution was complete. Irradiation (365 nm) was then carried out for 12 h and the solution was diluted with dichloromethane and washed with 1M sodium hydroxide. The combined organic layer was dried, concentrated and subjected to column chromatography in dichloromethane containing 2% methanol to give **79** (74 mg, 74%); $[\alpha]_D$ -33° (*c* 1.6, $CHCl_3$); ^{1}H NMR (600 Hz $CDCl_3$) δ 7.39-7.12 (30 H, m, Ar), 4.92 (d, 1 H, J_{gem} 10.8 Hz, OC$\underline{H}_2$Ph), 4.89 (s, 1 H, H-1'), 4.84 (d, 1 H, J_{gem} 12.3 Hz, OC$\underline{H}_2$Ph), 4.84 (d, 1 H, J_{gem} 10.4 Hz, OC$\underline{H}_2$Ph), 4.81 (d, 1 H, J_{gem} 11.9 Hz, OC$\underline{H}_2$Ph), 4.63 (d, 1 H, J_{gem} 12.1 Hz, OC$\underline{H}_2$Ph), 4.60 (d, 1 H, J_{gem} 12.0 Hz, OC$\underline{H}_2$Ph), 4.52 (d, 1 H, J_{gem} 10.8 Hz, OC$\underline{H}_2$Ph), 4.52 (d, 1 H, J_{gem} 12.1 Hz, OC$\underline{H}_2$Ph), 4.48 (d, 1 H, J_{gem} 11.6 Hz, OC$\underline{H}_2$Ph), 4.46 (d, 1 H, J_{gem} 11.8 Hz, OC$\underline{H}_2$Ph), 4.45 (d, 1 H, J_{gem} 10.8 Hz, OC$\underline{H}_2$Ph), 4.40 (d, 1 H, J_{gem} 11.8 Hz, OC$\underline{H}_2$Ph), 4.37 (s, 1 H, H-1), 4.36 (d, 1 H, d, $J_{2\text{-}3}$ 3.3 Hz, H-2), 4.27 (d, 1 H, $J_{2\text{-}3}$ 3.0 Hz, H-2'), 3.98 (dt, 1 H, J_{gem} 9.6, J_{vic} 6.2 Hz, OC$\underline{H}_2$CH$_2$), 3.91 (dd, 1 H, $J_{3\text{-}4}$≈$J_{4\text{-}5}$ 9.3 Hz, H-4), 3.80 (dd, 1 H,

J_{3-4}≈J_{4-5} 9.4 Hz, H-4'), 3.76 (1 H, dd, J_{gem} 10.6, J_{vic} 2.1 Hz, H-6a'), 3.74 (dd, 1 H, J_{gem} 10.2, J_{vic} 1.9 Hz, H-6a), 3.70 (dd, 1 H, J_{vic} 5.1 Hz, H-6b'), 3.66 (dd, 1 H, J_{vic} 5.5 Hz, H6b), 3.56-3.51 (3H, m, H-3, H-3', OCH_2CH_2), 3.44 (ddd, 1 H, H-5'), 3.39 (ddd, 1 H, H-5), 2.84-2.74 (ABMN 2 H, CH_2NH_2), 2.59-2.50 (ABMN, 2 H, $SCH_2CH_2NH_2$), 2.52 (t, 2 H, J 7.1 Hz, $OCH_2CH_2CH_2S$), 1.82 (2 H, p, J 6.6 Hz, $OCH_2CH_2CH_2S$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 138.2, 138.0, 137.9, 137.9, 137.9, 128.3-127.6, 100.8 ($^1J_{C-H}$ 156 Hz), 99.7 ($^1J_{C-H}$ 163 Hz), 81.4, 80.6, 75.6, 75.2, 75.2, 75.1, 74.2, 74.2, 73.5, 73.4, 71.4, 71.0, 70.7, 69.7, 69.2, 68.2, 40.6, 29.6, 29.2, 28.2; ES HRMS Calcd. for $C_{59}H_{70}NO_{11}S$ 1000.4669 Found 1000.4660.

*3-(2-Aminoethylthio)-propyl (3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside*. (**80**)

Allyl trisaccharide (65 mg) (**67**) was added to a quartz vessel and dissolved in a minimum amount of dichloromethane (400 µL). Methanol (5 mL) was then added followed by 2-aminoethanethiol hydrochloride (1.0 g). The reaction was stirred until dissolution was complete. Irradiation (365 nm) was carried out for 16 h and then the solution was diluted with dichloromethane and washed with 1M sodium hydroxide. The combined organic layer was dried, concentrated and subjected to column chromatography in dichloromethane containing 2% methanol to give **80** (47 mg, 72%); $[\alpha]_D$ –48.7° (*c* 1.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.46-6.89 (42 H, m, Ar), 5.15 (s, 1 H, H-1"), 5.08 (s, 1 H, H-1'), 4.92 (d, 3 H, J 10.5 Hz, OCH_2Ph), 4.70 (d, 1 H, J 11.3 Hz, OCH_2Ph), 4.66 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2'), 4.63 (d, 1 H, J 10.7 Hz, OCH_2Ph), 4.62 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.54 (d, 1 H, J 11.0 Hz, OCH_2Ph), 4.49 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.45 (d, 1 H, J 11.2 Hz, OCH_2Ph), 4.43-4.40 (m, 5 H, 4(OCH_2Ph), H-1), 4.37 (d, 1 H, J 12.0 Hz, OCH_2Ph), 4.30 (m, 2 H, J 11.9 Hz, 2(OCH_2Ph)), 4.26 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2"), 4.17 (d, 1 H, J 10.1 Hz, OCH_2Ph), 4.01-3.95 (m, 3 H, OCH_2Ph, OCH_2CH_2, H-4'), 3.82-3.58 (m, 10 H, H-4", H6a", H6b", H-3', H-6a', H-6b', H-3, H-4, H-6a, H-6b), 3.51 (dt, J_{vic} 6.9, J_{gem} 9.4 Hz, OCH_2CH_2), 3.47 (1 H, ddd, J_{4-5} 9.9, J_{5-6} 1.8, J_{5-6} 5.5 Hz, H-5'), 3.44 (dddd, 1 H, J_{4-5} 9.5, H_{5-6} 2.9, J_{5-6} 6.8 Hz, H-5), 3.40-3.37 (2 H, m, H-5", H-3"), 2.75 (2 H, t, J 6.8, CH_2NH_2), 2.54 (t, 2 H, J 6.8 Hz, $SCH_2CH_2NH_2$), 2.50 (t, 2 H, J 6.8 Hz, $OCH_2CH_2CH_2S$), 1.88-177 (2 H, m, $OCH_2CH_2CH_2S$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 138.2, 138.1, 137.9, 137.6, 137.2, 136.9, 136.4, 133.2, 133.0, 132.9, 129.0-127.5, 101.5 ($^1J_{C-H}$ 153 Hz), 99.3 ($^1J_{C-H}$ 163 Hz), 99.2 ($^1J_{C-H}$ 163 Hz), 83.7, 83.0, 80.4, 77.1, 75.4, 75.3, 75.1, 75.0, 74.8, 74.6, 74.1, 73.4, 73.3, 73.0, 72.6, 70.7, 70.4, 70.0, 69.8, 69.7, 68.7, 68.4, 40.7, 29.6, 28.6, 29.7; ES Calcd. 1536.5 found 1536.5 isotope pattern correct for Cl_3.

*3-(2-Aminoethylthio)-propyl (3,4,6-tri-*O*-p-chlorobenzyl-α-D-glucopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**81**).

Allyl trisaccharide (52 mg) (**74**) was added to a quartz vessel and dissolved in a minimum amount of dichloromethane (400 µL). Methanol (~5 mL) was then added to the vessel followed by 2-aminoethanethiol hydrochloride (1.0 g). The reaction was stirred until dissolution was complete. Irradiation 365 nm was carried out for 18 h and then the solution was diluted with dichloromethane and washed with 1M sodium hydroxide. The combined organic layer was dried, concentrated and subjected to column chromatography in dichloromethane containing 2% methanol to give **81** (40 mg, 76%); $[\alpha]_D$ –52.5° (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CHCl_3$) δ 7.41-6.81 (m, 42 H, Ar), 5.41 (d, J_{1-2} 3.8 Hz, H-1''), 4.96 (s, 1 H, H-1'), 4.93 (d, 1 H, J 10.8 Hz, OC$\underline{H}_2$Ph), 4.83 (d, 1 H, J 11.0 Hz, OC$\underline{H}_2$Ph), 4.82 (d, 1 H, J 11.0 Hz, OC$\underline{H}_2$Ph), 4.73 (d, 1 H, J 10.5 Hz, OC$\underline{H}_2$Ph), 4.70 (d, 1 H, J 12.0 Hz, OC$\underline{H}_2$Ph), 4.68 (d, 1 H, J 11.7 Hz, OC$\underline{H}_2$Ph), 4.61 (d, 1 H, J 11.4 Hz, OC$\underline{H}_2$Ph), 4.56-4.52 (m, 3 H, 3 (OC$\underline{H}_2$Ph)), 4.46 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 4.44 (d, 1 H, J 12.0 Hz, OC$\underline{H}_2$Ph), 4.42-4.34 (m, 6 H, H-1, H-2', 4 (OC$\underline{H}_2$Ph)), 4.29 (d, 1 H, J 11.6 Hz, OC$\underline{H}_2$Ph), 4.22 (ddd, 1 H, J_{4-5} 9.9, $J_{5-6}\approx J_{5-6}$ 2.0 Hz, H-5''), 4.18 (d, 1 H, J 10.6 Hz, OC$\underline{H}_2$Ph), 4.11 (d, 1 H, J 12.5 Hz, OC$\underline{H}_2$Ph), 3.96 (dt, 1 H, J_{gem} 9.6, J_{vic} 6.2 Hz, OC$\underline{H}_2$CHCH$_2$), 3.89 (dd, 1-H, $J_{3,4}\approx J_{4,5}$ 9.6 Hz, H-4'), 3.77 (dd, $J_{2-3}\approx J_{3-4}$ 9.2 Hz, H-3''), 3.73-3.55 (m, 12 H, H-2'', H-4'', H-6a'', H-6b'', H-3', H-6a', H-6b', H-3, H-4, H-6a, H-6b, OC$\underline{H}_2$CHCH$_2$), 3.46-3.40 (m, 2 H, H-5, H-5'), 2.80 (2 H, m, J 6.8 Hz, CH$_2$NH$_2$), 2.58 (2 H, m, SC$\underline{H}_2$CH$_2$NH$_2$), 2.52 (t, 2 H, J 7.4 Hz, OCH$_2$CH$_2$C$\underline{H}_2$S), 1.81 (p, 2 H, J 7.2 Hz, OCH$_2$C$\underline{H}_2$CH$_2$S); ^{13}C NMR (125 MHz, $CDCl_3$) 138.2, 137.9, 137.7, 137.6, 136.8, 136.6, 136.4, 136.4, 136.5, 133.2, 133.2, 132.8, 129.2-127.5, 101.7 ($^1J_{C-H}$ 154 Hz), 100.0 ($^1J_{C-H}$ 159.8 Hz), 99.9 ($^1J_{C-H}$ 171.8 Hz), 83.0, 80.7, 80.6, 75.0, 75.3, 75.2, 75.1, 74.9, 74.6, 74.5, 74.2, 74.2, 73.5, 72.7, 71.6, 70.2, 70.1, 70.0, 69.7, 69.3, 69.1, 68.7, 67.1, 40.2, 29.4, 29.4, 28.5 ES Calcd. 1536.5 found 1536.5 isotope pattern correct for Cl_3.

*3-(2-Aminoethylthio)-propyl (3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-p-chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**82**).

Allyl tetratrasaccharide (70 mg) (**75**) was added to a quartz vessel and dissolved in a minimum amount of dichloromethane (400 µL). Methanol (5 mL) was then added followed by 2-aminoethanethiol hydrochloride (1.0 g). The reaction was stirred until dissolution was complete. Irradiation 365 nm was carried out for 12 hrs and then the solution was diluted with dichloromethane and washed with 1M sodium hydroxide. The combined organic layer was dried,

concentrated and subjected to column chromatography in dichloromethane containing 2% methanol giving **82** (53 mg, 74%); $[\alpha]_D$ -51.7° (*c* 0.4, $CHCl_3$);^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45-6.89 (54 H, Ar), 5.48 (s, 1 H, H-1"), 5.16 (s, 1 H, H-1""), 5.08 (s, 1 H, H-1'), 4.88 (d, 1 H, J 10.3 Hz, OCH_2Ph), 4.87 (d, 1 H, J 11.0 Hz, OCH_2Ph), 4.81 (d, 1 H, J 10.4 Hz, OCH_2Ph), 4.81 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2'), 4.78 (d, 1 H, J 10.3 Hz, OCH_2Ph), 4.71 (d, 2 H, J 11.4 Hz, OCH_2Ph), 4.57 (d, 1 H, J 12.5 Hz, OCH_2Ph), 4.56 (d, 1 H, J 12.3 Hz, OCH_2Ph), 4.53 (d, 1 H, $J_{2,3}$ 2.7 Hz, H-2), 4.53 (d, 1 H, J 12.5 Hz, OCH_2Ph), 4.46 (d, 1 H, J 10.4 Hz, OCH_2Ph), 4.45 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2"), 4.45 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.39 (d, 1 H, J 11.4 Hz, OCH_2Ph), 4.38 (d, 2 H, J 11.5 Hz, OCH_2Ph), 4.38 (d, 1 H, J 12.5 Hz, OCH_2Ph), 4.37 (s, 1 H, H-1), 4.35 (d, 1 H, J 12.1 Hz, OCH_2Ph), 4.32 (d, 1 H, J 12.1 Hz, OCH_2Ph), 4.31 (d, 1 H, J 9.9 Hz, OCH_2Ph), 4.31 (d, 1 H, H2""), 4.31 (d, 1 H, J 11.1 Hz, OCH_2Ph), 4.29 (d, 1 H, J 10.3 Hz, OCH_2Ph), 4.25 (d, 1 H, J 12.3 Hz, OCH_2Ph), 4.07 (d, 1 H, J 11.9 Hz, OCH_2Ph), 3.98 (dt, 1 H, J_{gem} 9.5, J_{vic} 6.1 Hz, OCH_2CH_2), 3.91 (dd, 1 H, $J_{4,5}$=$J_{3,4}$ 9.3 Hz, H-4"), 3.89 (d, 1 H, J 11.9 Hz, OCH_2Ph), 3.83 (dd, 1 H $J_{4,5}$=$J_{3,4}$ 9.4 Hz, H-4""), 3.75-3.61 (12 H, H-4, H-6a, H-6b, H-3', H-4', H-6a', H-6b', H-6a", H-6b", H-6a"", H-6b"", OCH_2Ph), 3.59-3.57 (2 H, H-3, H-5"), 3.52-3.48 (2 H, H-5"", OCH_2CH_2), 3.47-3.41 (3 H, H-3", H-3"", H-5'), 3.34 (ddd, $J_{5,6a}$ 2.0, $J_{5,6b}$ 4.4, $J_{4,5}$ 9.7 Hz, H-5), 2.78 (t, 2 H, J_{vic} 6.4 Hz, CH_2NH_2), 2.53 (t, 2 H, J_{vic} 6.4, $SCH_2CH_2NH_2$), 2.50 (t, 2 H, J_{vic} 7.0, $OCH_2CH_2CH_2S$), 1.89-1.78 (m, 2 H, $OCH_2CH_2CH_2S$);^{13}C NMR (125 MHz, $CDCl_3$) δ 139.9, 138.7, 138.6, 138.4, 138.4, 138.3, 137.7, 137.5, 137.3, 137.3, 137.1, 137.0, 134.0, 134.0, 133.9, 133.8, 133.6, 129.9-128.4, 102.4($^1J_{C-H}$ 155 Hz, C1), 101.7 ($^1J_{C-H}$ 161 Hz, C1'), 101.1 ($^1J_{C-H}$ 164 Hz, C1""), 100.3 ($^1J_{C-H}$ 165 Hz, C1"), 83.6, 82.4, 81.4, 80.9, 76.3, 76.2, 75.9, 75.8, 75.8, 75.3, 75.1, 74.9,74.8, 74.8, 74.2, 74.1, 73.5, 73.4, 73.3, 70.8, 70.6, 70.4, 70.3, 70.1, 69.9, 69.7, 69.1, 69.0. 68.0, 39.4, 30.4, 29.7, 29.4, 28.8 EMS Calcd, 2072 Found (M+H) 2073, isotope intensities for Cl_6 correct.

3-(2-Aminoethylthio)-propyl (3,4,6-tri-O-p-*chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri*-O-p-*chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri*-O-p-*chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri*-O-p-*chlorobenzyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri*-O-*benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri*-O-*benzyl-β-D-mannopyranoside* (**83**)

Allyl hexasaccharide (64 mg) (**78**) was added to a quartz vessel and dissolved in a minimum amount of dichloromethane (1 mL). Methanol (5 mL) was then added followed by 2-aminoethanethiol hydrochloride (1.0 g). Irradiation (365 nm) was carried out for 14 h and then the solution was diluted with dichloromethane and washed with 1M sodium hydroxide. The combined organic layer was dried, concentrated and subjected to column chromatography in

dichloromethane containing 2% methanol giving **83** (49 mg, 75%); $[\alpha]_D$ -67° (*c* 0.3, $CHCl_3$); ^1H NMR (600 Hz $CDCl_3$) δ 7.43-6.59 (66 H, Ar), 5.57 (s, 1 H, H-1'''), 5.53 (s, 1 H, H-1''), 5.43 (s, 1 H, H-1''''), 5.10 (s, 1 H, H-1'''''), 5.06 (s, 1 H, H-1'), 4.89 (d, 1 H, J_{gem} 10.3 Hz, $OC\underline{H}_2Ph$), 4.88 (d, 1 H, J_{gem} 11.4 Hz, $OC\underline{H}_2Ph$), 4.86 (d, 1 H, J_{gem} 11.2 Hz, $OC\underline{H}_2Ph$), 4.80 (d, 1 H, J_{gem} 10.3 Hz, $OC\underline{H}_2Ph$), 4.79 (d, 1 H, J_{2-3} 3.4 Hz, H-2'), 4.69 (d, 1 H, J_{gem} 11.1 Hz, $OC\underline{H}_2Ph$), 4.69 (d, 1 H, J_{gem} 11.1 Hz, $OC\underline{H}_2Ph$), 4.62 (d, 1 H, J_{gem} 11.2 Hz, $OC\underline{H}_2Ph$), 4.59 (d, J_{2-3} 2.5 Hz, H-2'''), 4.59 (d, J_{2-3} 2.4 Hz, H-2''), 4.55 (d, 1 H, J 12.3 Hz, $OC\underline{H}_2Ph$), 4.54 (d, 1 H, J_{gem} 10.0 Hz, $OC\underline{H}_2Ph$), 4.52 (d, 1 H, J_{2-3} 3.5 Hz, H-2), 4.50 (d, 1 H, J_{gem} 12.3 Hz, $OC\underline{H}_2Ph$), 4.49-4.29 (m, 23 H, H-2 '''', H-1, 21($OC\underline{H}_2Ph$), 4.27 (d, 1 H, J_{2-3} 3.1 Hz, H-2''''), 4.24 (d, 1 H, J_{gem} 12.3 Hz, $OC\underline{H}_2Ph$), 4.22 (d, 1 H, J_{gem} 12.1 Hz, $OC\underline{H}_2Ph$), 4.18 (d, 1 H, J_{gem} 12.1 Hz, $OC\underline{H}_2Ph$), 4.08 (d, 1 H, J_{gem} 10.8 Hz, $OC\underline{H}_2Ph$), 4.08 (d, 1 H, J_{gem} 12.5 Hz, $OC\underline{H}_2Ph$), 3.95 (dt, 1 H, J_{gem} 9.5, J_{vic} 6.0 Hz, $OC\underline{H}_2CH_2CH_2$), 3.92 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.3 Hz, H-4''''), 3.87 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.3 Hz, H-4'''''), 3.84 (d, 1 H, J_{gem} 11.9 Hz, $OC\underline{H}_2Ph$), 3.79 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 10.0 Hz, H-4'), 3.77 (d, 1 H, J_{gem} 11.2 Hz, $OC\underline{H}_2Ph$), 3.75-3.54 (m, 19 H, H-3, H-4, H-6a, H-6b, H-3', H-6a', H-6b', H-4'', H-5'', H-6a'', H-6b'', H-4''' H-5''', H-6a''', H-6b''', H-6a'''', H-6b'''', H-6a''''', H6b''''', 2($OC\underline{H}_2Ph$)), 3.51 (ddd, 1 H, J_{4-5} 9.3, J_{5-6} 3.6, J_{5-6} 1.8 Hz, H-5'''''), 3.47 (dt, 1 H, $OC\underline{H}_2CH_2CH_2$), 3.43-3.39 (m, 5-H, H-3'', H-3''', H-3'''', H-5', H-5'''''), 3.36 (dd, 1 H, H-3''''), 3.31 (ddd, J_{4-5} 9.7, J_{5-6} 2.0, J_{5-6} 4.2 Hz, H-5), 2.74 (t, J 6.6 Hz, $SCH_2C\underline{H}_2NH_2$), 2.50 (t, 2 H, J_{vic} 7.0 Hz, $OCH_2CH_2C\underline{H}_2S$), 1.81-1.75 (m, 2 H, $OCH_2C\underline{H}_2CH_2S$); ^{13}C NMR (125 Hz, $CDCl_3$), 101.6 (J^1_{C-H} 154.0 Hz, C-1), 100.8 (J^1_{C-H} 162.3 Hz, C-1'), 100.2 (J^1_{C-H} 164.4 Hz, C-1'''), 100.1 (J^1_{C-H} 163.7 Hz, C-1''''), 100.1 (J^1_{C-H} 162.8 Hz, C-1), 99.7 (J^1_{C-H} 163.9 Hz, C-1'''') EMS Calcd. for $C_{165}H_{163}Cl_{12}O_{31}$ 3144.8 found 3144.9.

3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**84**).

The protected disaccharide (**79**) (55 mg) was dissolved in THF (2 mL) and *t*-butanol (2 mL). The solution was added in one portion to a solution of ammonia (~50 mL) and sodium metal (50 mg) stirred with a glass coated stir bar at –78 °C. The flask previously containing **79** was rinsed with THF (2 mL) and *t*-butanol (2 mL) this solution was added to the ammonia solution. After 30 minutes the reaction was quenched with methanol and the ammonia was allowed to evaporate at room temperature. The remaining THF was removed under vacuum and the resulting white solid was taken up in water (5 mL). The suspension was neutralized with a 5 M acetic acid solution against pH paper and filtered through a 0.2 μM filter. The solution was then passed through a C18 Sep-pac cartridge and eluted with methanol, to remove any compounds that would be irreversibly absorbed to the reverse phase silica. After concentration final purification by

HPLC on C18 silica was carried out to give a colourless glass **84** (21 mg, 85%); $[\alpha]_D$ -51° (*c* 0.3, H_2O); ^1H NMR (600 MHz, D_2O) δ 4.84 (s, 1 H, H-1'), 4.75 (s, 1 H, H-1), 4.27 (d, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 4.13 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2'), 3.99 (dt, 1 H, J_{gem} 10.1, J_{vic} 6.0 Hz, OCH_2CH_2), 3.94 (dd, 1 H, J_{gem} 12.3, $J_{5,6}$ 2.3 Hz, H-6a), 3.93 (d, 1 H, J_{gem} 12.5, $J_{5,6}$ 2.3 Hz, H-6a'), 3.77-3.72 (3H, m, H6b, H6b', OCH_2CH_2), 3.65 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.62 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3'), 3.59 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.2 Hz, H-4), 3.57 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.5 Hz, H-4'), 3.40 (ddd, 1 H, $H_{5,6}$ 6.6 Hz, H-5), 3.36 (ddd, 1 H, $J_{5,6}$ 6.8 Hz, H-5') 3.24 (t, 2 H, J_{vic} 6.6 Hz, $CH_2NH_3^+$), 2.88 (t, 2 H, J 6.7, $SCH_2CH_2NH_3^+$), 2.88 (t, 2 H, $OCH_2CH_2CH_2S$), 2.69 (t, 1 H, J 7.4 Hz, $CH_2CH_2CH_2S$), 1.39 (p, 2 H, J 7.3 Hz, $OCH_2CH_2CH_2S$); ^{13}C NMR (125 MHz, D_2O) δ 101.5 ($^1J_{C-H}$ 163 Hz, C-1'), 101.0 ($^1J_{C-H}$ 156.3 Hz, C-1"), 78.9, 77.3, 73.7, 73.1, 71.2, 69.3, 68.1, 67.6, 61.9, 61.8, 39.2, 29.2, 29.6, 28.5; ES HRMS Calcd. for $C_{17}H_{34}N_1O_{11}S$: 460.1852 found 460.1852.

3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**85**).

Same procedure as used for compound **84**. Compound **80** (40 mg) gives a colourless glass **85** (16 mg 78 %) $[\alpha]_D$ -52° (*c* 0.2, H_2O); ^1H NMR (600 MHz, D_2O) δ 4.96 (s, 1 H, H-1"), 4.91 (s, 1 H, H-1'), 4.73 (s, 1 H, H-1), 4.36 (d, 1 H, $J_{2,3}$ 2.7 Hz, H-2'), 4.24 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.15 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2"), 3.99 (dt, 1 H, J_{gem} 10.1 Hz, J_{vic} 6.1 Hz, OCH_2CH_2), 3.94 (dd, 1 H, J_{gem} 12.2 Hz, H-6a), 3.93 (d, 1 H, J_{gem} 12.0 Hz, H-6a"), 3.92 (d, 1 H, J_{gem} 11.8 Hz, H-6a'), 3.76 (dd, 1 H, $J_{5,6}$ 7.1 Hz, H-6b'), 3.74 (dd, 1 H, $J_{5,6}$ 6.5 Hz, H-6b"), 3.72 (d, 1 H, $J_{5,6}$ 6.5 Hz, H-6b), 3.74 (dt, 1 H, J_{vic} 6.0 Hz, OCH_2CH_2), 3.68 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3), 3.63 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3'), 3.62 (dd, 1 H, $J_{3,4}$ 10.7 Hz, H-3"), 3.60 (dd, 1 H, $J_{4,5}$ 9.0 Hz, H-4'), 3.57 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.6 Hz, H-4"), 3.49 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 10.2 Hz, H-4), 3.38 (ddd, 1 H, H-5"), 3.38 (ddd, 1 H, H-5), 3.37 (1H, ddd, H-5'), 3.24 (t, 2 H, J_{vic} 6.9 Hz, $CH_2NH_3^+$), 2.88 (t, 2 H, $SCH_2CH_2NH_3^+$), 2.73-2.65 (m, 2 H, $OCH_2CH_2CH_2S$), 1.97-1.91 (m, 2H, $OCH_2CH_2CH_2S$); ^{13}C NMR (125 MHz, D_2O) δ 101.9 ($^1J_{C-H}$ 161.1 Hz, C-1"), 101.6 ($^1J_{C-H}$ 162 Hz, C-1'), 101.0 ($^1J_{C-H}$ 159.1 Hz, C-1), 79.7, 78.3, 77.3, 77.2, 77.1, 73.8, 73.1, 72.9, 71.2, 69.3, 68.3, 67.8, 67.6, 62.0, 61.7, 61.7, 39.2, 29.6, 29.2, 28.6 ES HRMS Calcd. for $C_{23}H_{44}N_1O_{16}S$ 622.2381 found 622.2384.

3-(2-Aminoethylthio)-propyl (α-D-glucopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**86**).

Same procedure as used for compound **84**. Compound **81** (35 mg) gives a colourless glass **86** (16 mg 82%) $[\alpha]_D$ –0.5° (*c* 0.2, H_2O); ^1H NMR (600 MHz, D_2O) δ 5.45 (1 H, d, $J_{1,2}$ 3.8

Hz, H-1"), 4.88 (s, 1 H, H-1'), 4.74 (s, 1 H, H-1), 4.35 (s, 1 H, H-2'), 4.26 (d, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 4.04 (ddd, 1 H, $J_{5,6}$ 2.2, $J_{5,6}$ 4.9, $J_{4,5}$ 10.2 Hz, H-5"), 3.99 (dt, 1 H, J_{gem} 10.1, J_{vic} 6.1 Hz, $OC\underline{H}_2CH_2$), 3.95 (dd, 1 H, J_{gem} 12.4, J_{vic} 2.3 Hz, H-6a), 3.94 (d, 1 H, J_{gem} 12.4, J_{vic} 2.1 Hz, H-6a'), 3.89 (d, 1 H, J_{gem} 12.5, J_{vic} 2.3 Hz, H-6a"), 3.83 (dd, 1 H, $J_{2,3}\approx J_{3,4}$ 9.5 Hz, H-3"), 3.78 (1 H, dd, $J_{5,6}$ 7.4 Hz, H-6b'), 3.74-3.71 (m, 4 H, H-6b, H-3', H-4', H-6b", $OC\underline{H}_2CH_2$), 3.65 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.57 (dd, 1 H, H-2"), 3.48 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.45 (dd, 1 H, H-4"), 3.35 (ddd, 1 H, H-5'), 3.34 (ddd, 1 H, H-5), 3.24 (t, 2 H, J_{vic} 6.9 Hz, $CH_2NH_3^+$), 2.88 (t, 2 H, $SCH_2CH_2NH_3^+$), 2.70 (t, 2 H, J 7.3 Hz, $OCH_2CH_2C\underline{H}_2S$), 1.94 (p , 2 H, J 7.2 Hz, $OCH_2C\underline{H}_2CH_2S$); ^{13}C NMR (125 MHz, D_2O) δ 101.5 ($^1J_{C\text{-}H}$ 161.2 Hz, C1"), 101.1 ($^1J_{C\text{-}H}$ 159.3 Hz, C1'), 99.2 ($^1J_{C\text{-}H}$ 173.6 Hz, C1), 79.1, 77.8, 77.5, 75.6. 75.1, 73.5, 73.0, 72.9, 72.8, 70.2, 69.5, 68.8, 67.8, 62.1, 61.7, 61.6, 39.2, 29.5, 29.2, 28.5 ES HRMS Calcd. for $C_{23}H_{44}N_1O_{16}S$ 622.2381 found 622.2384.

3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**87**)

Same procedure as used for compound **84**. Compound **82** (56 mg) gives a colourless glass **87** (16 mg, 78%); $[\alpha]_D$ -53° (*c* 0.15, H_2O); ^1H NMR (600 MHz, D_2O) δ 5.03 (s, 1 H, H-1"), 4.93 (s, 1 H, H-1'"), 4.88 (s, 1 H, H-1'), 4.72 (s, 1 H, H-1), 4.39 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2"), 4.32 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2'), 4.24 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 4.15 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2'"), 3.99 (dt, 1 H, J_{gem} 10.1 Hz, J_{vic} 6.2, $OC\underline{H}_2CH_2$), 3.93 (d, 1 H, J_{gem} 12.3 Hz, H-6a'"), 3.93 (d, 1 H, J_{gem} 12.5 Hz, H-6a'), 3.92 (d, 1 H, J_{gem} 12.3 Hz, H-6a), 3.92 (d, 1 H, J_{gem} 12.3 Hz, H-6a"), 3.75 (d, 1 H, $J_{5,6}$ 6.2 Hz, H-6b"), 3.73 (d, 1 H, $J_{5,6}$ 6.7 Hz, H-6b), 3.73 (d, 1 H, $J_{5,6}$ 6.9 Hz, H-6b'"), 3.73 (1 H, dt, J_{vic}, 6.2 Hz, $OC\underline{H}_2CH_2$), 3.71 (d, 1 H, $J_{5,6}$ 5.7 Hz, H-6b'), 3.67 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.65 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3'), 3.62 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3"), 3.60 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3'"), 3.59 (dd, 1 H, $J_{3,4}\approx J_{4,5}$ 9.8 Hz, H-4"), 3.56 (dd, 1 H, $J_{3,4}\approx J_{4,5}$ 9.5 Hz, H-4'"), 3.50 (dd, 1 H, $J_{3,4}\approx J_{4,5}$ 9.7 Hz, H-4'), 3.47 (dd, 1 H, $J_{3,4}\approx J_{4,5}$ 9.7 Hz, H-4), 3.39-3.34 (m, 4-H, H-5, H-5', H-5", H-5'"), 3.24 (t, 2 H, J_{vic} 6.6 Hz, $CH_2NH_3^+$), 2.87 (t, 2 H, $SCH_2CH_2NH_3^+$), 2.69-2.67 (m, 2 H, $OCH_2CH_2C\underline{H}_2S$), 1.94-1.91 (m, 2H, $OCH_2C\underline{H}_2CH_2S$); ^{13}C NMR (125 MHz, D_2O) δ 101.9 ($^1J_{C\text{-}H}$ 163 Hz, C1"), 101.8 ($^1J_{C\text{-}H}$ 163 Hz, C1'"), 101.6 ($^1J_{C\text{-}H}$ 163 Hz, C1'), 100.8 ($^1J_{C\text{-}H}$ 160 Hz, C1"), 79.7 (C2"), 79.7 (C2'), 79.0 (C2), 77.1, 76.9, 76.9, 76.8 (C-5, C-5', C-5", C5'"), 73.7 (C3'"), 73.0 (C3"), 72.6 (C3'), 72.6 (C3), 71.1 (C2'"), 69.1 ($O\underline{C}H_2CH_2$), 68.2 (C4), 67.8 (C4'), 67.6 (C4"), 67.5 (C4'"), 61.9, 61.5, 61.4, 61.3 (C6, C6', C6", C6'"), 39.0($\underline{C}H_2NH_3^+$), 29.3 ($S\underline{C}H_2CH_2NH_3^+$), 28.9 ($OCH_2CH_2C\underline{H}_2S$), 28.2 ($OCH_2\underline{C}H_2$).

3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**88**).

Same procedure as used for compound **84**. Compound **83** (25 mg) gives a colourless glass **88** (7 mg 83 %); ^{1}H NMR (600 MHz D_2O) δ 5.06 (1 H, s, H-1''''), 5.02 (2 H, s, H-1'', H-1'''), 4.97 (1 H, s, H-1'''''), 4.90 (1 H, s, H-1'), 4.74 (1 H, s, H-1), 4.41 (1 H, d, J_{2-3} 4.4 Hz, H-2''), 4.40 (1 H, d, J_{2-3} 3.8 Hz, H-2''''), 4.37 (1 H, d, J_{2-3} 3.3 Hz, H-2'''), 4.34 (1 H, d, J_{2-3} 3.3 Hz, H-2'), 4.25 (1 H, d, J_{2-3} 3.1 Hz, H-2), 4.16 (1 H, d, J_{2-3} 3.3 Hz, H-2'''''), 4.00 (1 H, dt, J_{vic} 6.0, J_{gem} 10.1 Hz, OC<u>H</u>$_2$CH$_2$), 3.95 (1 H, dd, J_{vic}<2.5, J_{gem} 11.6 Hz, H-6a'''), 3.94 (1 H, dd, J_{vic}<2.5, J_{gem} 11.5 Hz, H-6a), 3.94 (1 H, dd, J_{vic}<2.5, J_{gem} 11.9 Hz, H-6a'), 3.94 (1 H, dd, J_{vic}<2.5, J_{gem} 12.0 Hz, H-6a''''), 3.93 (1 H, dd, J_{vic}<2.5, J_{gem} 11.6 Hz, H-6a''), 3.93 (1 H, dd, J_{vic}<2.5, J_{gem} 11.6 Hz, H-6a'''''), 3.76 (1 H, dd, J_{vic} 6.4 Hz, H-6b''''), 3.75 (1 H, dd, J_{5-6} 6.4 Hz, H-6b''), 3.74 (1 H, dd, J_{5-6} 8.1 Hz, H-6b'''''), 3.75 (1 H, dd, J_{5-6} 6.2 Hz, H-6b), 3.74 (1 H, dd, J_{5-6} 6.2 Hz, H-6b'), 3.72 (1 H, dd, J_{5-6} 6.7 Hz, H-6b'''), 3.69 (1 H, dd, J_{3-4} 10.3 Hz, H-3), 3.67 (1 H, dd, J_{3-4} 10.4 Hz, H-3'), 3.67 (1 H, dd, J_{3-4} 9.3 Hz, H-3''), 3.67 (1 H, dd, J_{3-4} 9.3 Hz, H-3'''), 3.65 (1 H, dd, J_{3-4} 11.0 Hz, H-3''''), 3.63 (1 H, dd, J_{3-4} 10.0 Hz, H-3'''''), 3.60 (1 H, dd, J_{4-5} 10.0 Hz, H-4''), 3.58 (1 H, dd, J_{4-5} 10.0 Hz, H-4''''), 3.51 (1 H, dd, J_{4-5} 10.0 Hz, H-4'''''), 3.51 (1 H, dd, J_{4-5} 10.0 Hz, H-4'''), 3.51 (1 H, dd, J_{4-5} 10.0 Hz, H-4'), 3.48 (1 H, dd, J_{4-5} 10.0 Hz, H-4), 3.43-3.35 (6H, m, H-5'''', H-5''''', H-5''', H-5', H-5, H-5''), 3.23 (1 H, t, J_{vic} 6.6, SCH$_2$C<u>H</u>$_2$NH$_3^+$), 2.84 (1 H, t, SC<u>H</u>$_2$CH$_2$NH$_3^+$), 2.71-2.68 (2H, m, CH$_2$CH$_2$C<u>H</u>$_2$S), 1.96-1.92 (2 H, m, CH$_2$C<u>H</u>$_2$CH$_2$); ^{13}C NMR (125 MHz, $CDCl_3$), 102.1 (C-1', C-1'',C-1'''), 101.9 (C-1''''), 101.7 (C-1'''''), 101,0 (C-1), 80.0, 80.0, 79.3(C-2, C-2'', C-2', C-2'''), 79.3 (C-2''''), 77.1-76.8 (C-5, C-5', C-5'', C-5''', C-5'''', C-5'''''), 73.8 (C-3''''), 73.1-72.7 (C-3, C-3',C-3'', C-3''', C-3''''), 71.3 (C-2'''''), 69.3 (O<u>C</u>H$_2$CH$_2$), 68.5, 68.1, 68.0, 68.1, 68.0, 67.9, 67.7 (C-4, C-4', C-4'', C-4''', C-4'''', C-4'''''), 62.1-61.2 (C-6, C-6', C-6'', C-6''', C-6'''', C-6'''''), 39.3 (SCH$_2$<u>C</u>H$_2$NH$_3^+$), 29.6 (S<u>C</u>H$_2$CH$_2$NH$_3^+$), 29.4 (CH$_2$CH$_2$<u>C</u>H$_2$S), 28.6(CH$_2$<u>C</u>H$_2$CH$_2$); ES HRMS Calcd. for $C_{41}H_{74}NO_{31}S$ 1108.3966 found 1108.3956.

Propyl β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**76**).

The protected disaccharide (**67**)(106 mg) was dissolved in an EtOH:toluene (10:1) solution and palladium on carbon (10%)(5 mg) was added. The flask was sealed and a hydrogen balloon attached. The solution was stirred for 48 h and then filtered through celite. After concentration final purification by HPLC on C18 silica was carried out to give a colourless glass **76** (36 mg, 82%); ^{1}H NMR (500 MHz D_2O) δ 4.82 (1 H, s, H-1'), 4.71 (1 H, s, H-1), 4.23 (1 H, d, J_{2-3} 3.1 Hz, H-2), 4.12 (1 H, d, J_{2-3} 3.2 Hz, H-2'), 3.91 (1 H, d, J_{vic} 2.1, J_{gem} 12.2 Hz, H-6), 3.91 (1

H, d, J_{vic} 2.1, J_{gem} 12.2 Hz, H-6a'), 3.83 (1 H, dt, J_{vic} 6.6, J_{gem} 9.6 Hz, $OC\underline{H}_2CH_2$), 3.76 (1 H, dd, J_{5-6} 6.7, J_{gem} 12.3 Hz, H-6b'), 3.76 (1 H, dd, J_{5-6} 6.7, J_{gem} 12.3 Hz, H-6b), 3.64-3.60 (3 H, m, H-3, H-3', $OC\underline{H}_2CH_2$), 3.56 (1 H, dd, J_{3-4}≈J_{4-5} 9.5 Hz, H-4), 3.54 (1 H, dd, J_{3-4}≈J_{4-5} 9.7 Hz, H-4'), 3.39-3.32 (2 H, m, H-5, H-5'), 1.60 (2 H, p, J 7.0 Hz, $OCH_2C\underline{H}_2CH_3$), 0.90 (3 H, t, $CH_2CH_2C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$), 101.5 (J^1_{C-H} 162.0 Hz, C-1'), 100.9 (J^1_{C-H} 158.5 Hz, C-1), 79.0 (C-2), 77.2, 77.2 (C-5, C-5'), 73.6, 73.1 (C-3, C3'), 72.7 ($O\underline{C}H_2CH_2$), 71.2 (C-2'), 68.1, 67.6 (C-4',C-4), 61.9-61.8 (C-6, C-6', C-6"), 23.2 ($CH_2\underline{C}H_2CH_3$), 10.8 ($CH_2\underline{C}H_3$); ES HRMS Calcd. for $C_{21}H_{38}O_{16}Na$ 384.2058.

Propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**89**).

The protected allyl trisaccharide (**72**) (40 mg) was dissolved in THF:ethanol (1:1, 4 mL) solution and hydrazine hydrate (400 μL) was added. The reaction was stirred vigorously uncovered for 3 h. The reaction was then diluted with toluene and washed repeatedly with water. Concentration gave a colourless syrup which showed no allyl resonances in the ^1H NMR. This syrup was dissolved in THF (2 mL) and *t*-butanol (2 mL). The solution was added in one portion to a solution of ammonia (~50 mL) and sodium metal (50 mg) stirred with a glass coated stir bar at –78 °C. The flask previously containing **72** was rinsed with THF (2 mL) and *t*-butanol (2 mL) this solution was added to the ammonia solution. After 30 minutes the reaction was quenched with methanol and the ammonia was allowed to evaporate at room temperature. The remaining THF was removed under vacuum and the resulting white solid was taken up in water (5 mL). The suspension was neutralized with a 5 M acetic acid solution against pH paper and filtered through a 0.2 μM filter. The solution was then passed through a C18 Sep-Pac cartridge and eluted with methanol, to remove any compounds that would be irreversibly absorbed to the reverse phase silica. After concentration final purification by HPLC on C18 silica was carried out to give a colourless glass **89** (11 mg, 84%) $[\alpha]_D$ -69° (c 0.4, H_2O); ^1H NMR (600 MHz D_2O) δ 4.95 (1 H, s, H-1"), 4.91 (1 H, s, H-1'), 4.72 (1 H, s, H-1), 4.37 (1 H, d, J_{2-3} 3.3 Hz, H-2'), 4.23 (1 H, d, J_{2-3} 3.1 Hz, H-2), 4.15 (1 H, d, J_{2-3} 3.3 Hz, H-2"), 3.93 (1 H, dd, J_{vic}<2.5 Hz, H-6a), 3.92 (1 H, dd, J_{vic}<2.5 Hz, H6a"), 3.93 (1 H, dd, J_{vic}<2.5 Hz, H-6a'), 3.86 (1 H, dt, J_{vic} 6.6, J_{gem} 9.7 Hz, $OC\underline{H}_2CH_2$), 3.76 (1 H, dd, J_{5-6} 6.2, J_{gem} 13.6 Hz, H-6b'), 3.73 (1 H, dd, J_{5-6} 6.8, J_{gem} 12.4 Hz, H-6b"), 3.71 (1 H, dd, J_{5-6} 5.9, J_{gem} 11.9 Hz, H-6b), 3.67 (1 H, dd, J_{3-4} 9.9 Hz, H-3), 3.64 (1 H, dd, J_{3-4} 9.7 Hz, H-3'), 3.61 (1 H, dd, J_{3-4} 9.0 Hz, H-3"), 3.59 (1 H, dt, J_{vic} 7.1 Hz, $OC\underline{H}_2CH_2$), 3.59 (1 H, dd, J_{4-5} 9.5 Hz, H-4'), 3.57 (1 H, dd, J_{4-5} 9.7 Hz, H-4"), 3.48 (1 H, dd, J_{4-5} 9.7 Hz, H-4), 3.38 (1 H, ddd, H-5'), 3.37 (1 H, ddd, H-5"), 3.37 (1 H, dd, H-5), 1.62 (2 H, p, J 7.1 Hz, $OCH_2C\underline{H}_2CH_3$), 0.91 (3 H, t, $CH_2CH_2C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$), 101.9 (J^1_{C-H} 162.0 Hz, C-1'), 101.6 (J^1_{C-}

$_H$ 162.2 Hz, C-1"), 100.9 (J^1_{C-H} 158.8 Hz, C-1), 79.7 (C-2), 79.0 (C-2'), 77.2, 77.2, 77.1 (C-5, C-5', C-5"), 73.8 (C-4'), 73.1 (C-3'), 73.0 (C-3"), 72.6 (OCH_2CH_2), 71.3 (C-2"), 68.3 (C-4), 67.8 (C-4'), 67.6 (C-4"), 62.0-61.6 (C-6, C-6', C-6"), 23.3 ($CH_2\underline{C}H_2CH_3$), 10.8 ($CH_2\underline{C}H_3$); ES HRMS Calcd. for $C_{21}H_{38}O_{16}Na$ 569.2058 found 569.2053.

Propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**90**).

Same procedure as was used for compound **89**. Compound **75** (52 mg) gives a colourless glass **90** (14 mg 78%); $[\alpha]_D$ -70° (*c* 0.4, H_2O); ^{1}H NMR (600 MHz D_2O) δ 5.04 (1 H, s, H-1"), 4.94 (1 H, s, H-1'"), 4.89 (1 H, s, H-1'), 4.73 (1 H, s, H-1), 4.40 (1 H, d, J_{2-3} 3.1 Hz, H-2"), 4.35 (1 H, d, J_{2-3} 3.1 Hz, H-2'), 4.24 (1 H, d, J_{2-3} 3.1 Hz, H-2), 4.16 (1 H, d, J_{2-3} 3.1 Hz, H-2'"), 3.94 (1 H, dd, J_{gem} 12.2, J_{vic} <2.5 Hz, H-6a'), 3.94 (1 H, dd, J_{gem} 12.2, J_{vic}<2.5 Hz, H-6a"), 3.92 (1 H, dd, J_{gem} 11.8, J_{vic}<2.5 Hz, H-6a), 3.92 (1 H, dd, J_{gem}11.9, J_{vic} <2.5 Hz, H-6a'"), 3.87 (1 H, dt, J_{vic} 6.8, J_{gem} 9.7 Hz, $OC\underline{H}_2CH_2$), 3.76 (1 H, dd, J_{5-6} 8.0 Hz, H-6b"), 3.74 (1 H, dd, J_{5-6} 8.0 Hz, H-6b'"), 3.74 (1 H, dd, J_{5-6} 6.5 Hz, H-6b), 3.72 (1 H, dd, J_{5-6} 7.1 Hz, H-6b'), 3.67 (1 H, dd, J_{3-4} 9.6 Hz, H-3), 3.68 (1 H, dd, J_{3-4} 9.5 Hz, H-3'), 3.64 (1 H, dd, J_{3-4} 10.8 Hz, H-3"), 3.62 (1 H, dd, J_{3-4} 10.8 Hz, H-3'"), 3.61 (1 H, dt, J_{vic} 6.8 Hz, $OC\underline{H}_2CH_2$), 3.60 (1 H, dd, J_{4-5} 10.1 Hz, H-4"), 3.57 (1 H, dd, J_{4-5} 9.5 Hz, H-4'"), 3.50 (1 H, dd, J_{4-5} 9.9 Hz, H-4'), 3.48 (1 H, dd, J_{4-5} 9.9 Hz, H-4), 3.39 (1 H, ddd, H-5'"), 3.39 (1 H, ddd, H-5'), 3.38 (1 H, ddd, H-5"), 3.38 (1 H, ddd, H-5), 1.63 (2 H, p, J 7.0 Hz, $OCH_2\underline{C}H_2CH_3$), 0.93 (3 H, t, $CH_2CH_2\underline{C}H_3$); ^{13}C NMR (125 MHz, $CDCl_3$), 102.0 (J^1_{C-H} 161.9 Hz, C-1'), 102.0 (J^1_{C-H} 162.3 Hz, C-1"), 101.8 (J^1_{C-H} 163.9 Hz, C-1'"), 101.8 (J^1_{C-H} 158.9 Hz, C-1), 80.1 (C-2), 79.9 (C-2'), 79.2 (C-2"), 77.1, 77.1, 77.0, 77.0 (C-5, C-5', C-5", C-5'"), 73.8 (C-3'"), 73.2 (C-3"), 72.8, 72.8 (C-3, C-3'), 72.6 ($O\underline{C}H_2CH_2$), 71.3 (C-2'"), 68.5 (C-4), 68.0 (C-4'), 67.8 (C-4"), 67.6 (C-4'"), 62.1-61.5 (C-6, C-6', C-6", C-6'"), 23.3 ($CH_2\underline{C}H_2CH_3$), 10.8 ($CH_2\underline{C}H_3$); ES HRMS Calcd. for $C_{27}H_{48}O_{21}Na$ 731.2586 found 731.2588.

Propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**91**)

Same procedure as was used for compound **89**. Compound **77** (32 mg) gives a colourless glass **91** (8 mg 77 %); $[\alpha]_D$ –22° (*c* 0.2, H_2O); ^{1}H NMR (800 MHz D_2O) δ 5.03 (1 H, s, H-1'"), 5.00 (1 H, s, H-1"), 4.95 (1 H, s, H-1'""), 4.89 (1 H, s, H-1'), 4.72 (1 H, s, H-1), 4.38 (1 H, d, J_{2-3} 3.7 Hz, H-2"), 4.38 (1 H, d, J_{2-3} 3.7 Hz, H-2'"), 4.34 (1 H, d, J_{2-3} 3.4 Hz, H-2'), 4.23 (1 H, d, J_{2-3} 3.4 Hz, H-2), 4.15 (1 H, d, J_{2-3} 3.4 Hz, H-2'""), 3.92 (1 H, dd, J_{gem}10.1, J_{vic} 2.0 Hz, H-6a'""), 3.92 (1 H, dd, J_{gem} 10.7, J_{vic}2.0 Hz, H-6a'), 3.92 (1 H, dd, J_{gem} 10.7, J_{vic}2.0 Hz, H-6a"), 3.92 (1 H, dd,

J_{gem} 10.9, J_{vic}2.2 Hz, H-6a), 3.92 (1 H, dd, J_{gem} 10.9, J_{vic}2.2 Hz, H-6a'''), 3.85 (1 H, dt, J_{vic} 6.6, J_{gem} 9.8 Hz, OC$\underline{H}_2$CH$_2$), 3.74 (1 H, dd, J_{5-6} 6.5 Hz, H-6b'''), 3.73 (1 H, dd, J_{5-6} 5.6 Hz, H-6b), 3.73 (1 H, dd, J_{5-6} 6.2 Hz, H-6b''''), 3.72 (1 H, dd, J_{5-6} 5.9 Hz, H-6b'), 3.72 (1 H, dd, J_{5-6} 5.6 Hz, H-6b''), 3.68 (1 H, dd, J_{3-4} 9.5 Hz, H-3), 3.67 (1 H, dd, J_{3-4} 9.8 Hz, H-3'), 3.66 (1 H, dd, J_{3-4} 9.7 Hz, H-3''), 3.63 (1 H, dd, J_{3-4} 9.7 Hz, H-3'''), 3.62 (1 H, dd, J_{3-4} 9.3 Hz, H-3''''), 3.60 (1 H, dt, J_{vic} 7.5 Hz, OC$\underline{H}_2$CH$_2$), 3.58 (1 H, dd, J_{4-5} 9.5 Hz, H-4'''), 3.56 (1 H, dd, J_{4-5} 9.5 Hz, H-4''''), 3.51 (1 H, dd, J_{4-5} 9.5 Hz, H-4''), 3.49 (1 H, dd, J_{4-5} 9.5 Hz, H-4'), 3.48 (1 H, dd, J_{4-5} 9.5 Hz, H-4), 3.39 (1 H, ddd, H-5''''), 3.39 (1 H, ddd, H-5'''), 3.37 (1 H, ddd, H-5'), 3.36 (1 H, ddd, H-5''), 3.36 (1 H, ddd, H-5), 1.62 (2 H, p, J 7.0 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 0.93 (3 H, t, CH$_2$CH$_2$C$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$), 102.2 (J^1_{C-H} 161.9 Hz, C-1''), 102.1 (J^1_{C-H} 162.3 Hz, C-1'), 101.9 (J^1_{C-H} 162.9 Hz, C-1'''), 101.8 (J^1_{C-H} 162.3 Hz, C-1''''), 100.8 (J^1_{C-H} 159.3 Hz, C-1), 80.1 (C-2, C-2'), 79.8 (C-2''), 79.3 (C-2'''), 77.1, 77.1, 77.0, 77.0, 77.0 (C-5, C-5', C-5'', C-5'''. C-5''''), 73.8 (C-3''''), 73.2, 72.9, 72.9, 72.7 (C-3, C-3', C-3'', C-3'''), 71.3 (C-2'''), 71.2 (O$\underline{C}$H$_2$CH$_2$), 68.5, 68.1, 67.9, 67.6 (C-4, C-4', C-4'', C-4''', C-4''''), 62.0, 61.7, 61.7, 61.5, 61.5 (C-6, C-6', C-6'', C-6''', C-6''''), 23.3 (CH$_2\underline{C}$H$_2$CH$_3$), 10.8 (CH$_2\underline{C}$H$_3$); ES HRMS Calcd. for $C_{33}H_{58}O_{26}Na$ 893.3114 found 893.3110.

Propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**92**).

Same procedure as was used for compound **89**. Compound **78** (45 mg) gives a colourless glass **92** (11 mg 81 %); $[\alpha]_D$ -75° (c 0.15, H_2O); ^{1}H NMR (600 MHz D_2O) δ 5.06 (1 H, s, H-1''''), 5.02 (2 H, s, H-1'', H-1'''), 4.96 (1 H, s, H-1'''''), 4.90 (1 H, s, H-1'), 4.77 (1 H, s, H-1), 4.40 (1 H, d, J_{2-3} 3.3 Hz, H-2''), 4.40 (1 H, d, J_{2-3} 3.5 Hz, H-2''''), 4.37 (1 H, d, J_{2-3} 3.5 Hz, H-2'''), 4.34 (1 H, d, J_{2-3} 3.3 Hz, H-2'), 4.24 (1 H, d, J_{2-3} 3.1 Hz, H-2), 4.16 (1 H, d, J_{2-3} 3.3 Hz, H-2'''''), 3.94 (1 H, dd, H-6a'''), 3.93 (1 H, dd, H-6a''''), 3.93 (1 H, dd, H-6a), 3.93 (1 H, dd, H-6a'), 3.92 (1 H, dd, H-6a''''), 3.92 (1 H, dd, H-6a''), 3.86 (1 H, dt, J_{vic} 6.6, J_{gem} 9.7, OC$\underline{H}_2$CH$_2$), 3.75 (1 H, dd, J_{5-6} 7.2, J_{gem} 13.3 Hz, H-6b''''), 3.73 (1 H, dd, J_{5-6} 6.5, J_{gem} 10.8 Hz, H-6b''''), 3.73 (1 H, dd, J_{5-6} 6.8, J_{gem} 13.0 Hz, H-6b), 3.73 (1 H, dd, J_{5-6} 6.5, J_{gem} 13.4 Hz, H-6b'), 3.73 (1 H, dd, J_{5-6} 6.1, J_{gem} 12.0 Hz, H-6b''), 3.72 (1 H, dd, J_{5-6} 6.1, J_{gem} 12.0 Hz, H-6b'''), 3.68 (1 H, dd, J_{3-4} 10.8 Hz, H-3), 3.68 (1 H, dd, J_{3-4} 9.0 Hz, H-3'), 3.67 (1 H, dd, J_{3-4} 10.5 Hz, H-3''), 3.67 (1 H, dd, J_{3-4} 9.0 Hz, H-3'''), 3.65 (1 H, dd, J_{3-4} 10.6 Hz, H-3''''), 3.63 (1 H, dd, J_{3-4} 10.1 Hz, H-3'''''), 3.61 (1 H, dt, J_{vic} 7.3 Hz, OCH$_2$CH$_2$), 3.59 (1 H, dd, J_{4-5} 9.9 Hz, H-4''''), 3.57 (1 H, dd, J_{4-5} 10.1 Hz, H-4'''''), 3.51 (1 H, dd, J_{4-5} 10.1 Hz, H-4''), 3.50 (1 H, dd, J_{4-5} 9.8 Hz, H-4'''), 3.50 (1 H, dd, J_{4-5} 9.4 Hz, H-4'), 3.48 (1 H, dd, J_{4-5} 10.5 Hz, H-4), 3.41 (1 H, ddd, H-5''''), 3.39 (1 H, ddd, H-5'''''), 3.40 (1 H, ddd, H-5'''), 3.38 (1 H, ddd, H-5'), 3.37 (1 H, ddd, H-5), 3.37 (1 H, ddd, H-5''), 1.63 (2 H, p, J 7.0 Hz,

$OCH_2\underline{C}H_2CH_3$), 0.92 (3 H, t, $CH_2CH_2\underline{C}H_3$); ^{13}C NMR (125 MHz, $CDCl_3$), 102.1 ($J^1_{C\text{-}H}$ 161.0 Hz, C-1'), 102.1 ($J^1_{C\text{-}H}$ 162.3 Hz, C-1",C-1'"), 101.9 ($J^1_{C\text{-}H}$ 162.1 Hz, C-1""), 101.8 ($J^1_{C\text{-}H}$ 162.0 Hz, C-1"""), 100.8 ($J^1_{C\text{-}H}$ 158.4 Hz, C-1), 80.0(C-2, C-2"), 80.0 (C-2'), 79.9 (C-2'"), 79.3 (C-2""), 77.1-76.8 (C-5, C-5', C-5", C-5'", C-5"", C-5"""), 73.8 (C-3"""), 73.1-72.7 (C-3, C-3',C-3", C-3'", C-3""), 72.6 ($O\underline{C}H_2CH_2$), 71.3 (C-2"""), 68.5, 68.1, 68.0, 67.9, 67.6 (C-4, C-4', C-4", C-4'", C-4"", C-4"""), 62.0-61.4 (C-6, C-6', C-6", C-6'", C-6"", C-6"""), 23.3 ($CH_2\underline{C}H_2CH_3$), 10.8 ($CH_2\underline{C}H_3$); ES HRMS Calcd. for $C_{39}H_{68}O_{31}Na$ 1055.3642 found 1055.3648.

4,6-O-benzylidene-3-O-benzyl-D-glucopyranose (**93**).

3-*O*-Benzyl-D-glucopyranose (3.0 g, 110 mmol) was dissolved in DMF (20 mL) and benzaldehyde dimethyl acetal (2.0 mL, 130 mmol) followed by *p*TSA (110 mg) were added. The solution was subjected to rotary evaporation for 2 h at 25 °C with a standard water aspirator. The solution was quenched with pyridine (200 μL) and concentrated to a yellow oil under vacuum. The oil was chromatographed on silica gel in toluene:EtOAc (1:1) to yield a white solid (3.9 g, 94%) after concentration. This solid could be recrystallized from EtOAc hexane-mixtures. mp. 148-149 °C; $[\alpha]_D$ –20.5° (c 1.1, $CHCl_3$); ^1H NMR (500 MHz, CH_3OD) δ 7.45-7.20 (m, 10 H, Ar), 5.59 (s, 1 H, $O_2C\underline{H}Ph$, β anomer), 5.58 (s, 1 H, $O_2C\underline{H}Ph$, α anomer), 5.13 (d, 1 H, $J_{1\text{-}2}$ 3.7 Hz, H-1, α anomer), 4.87-4.80 (m, 4 H, 4($OC\underline{H}_2Ph$)), 4.61 (d, 1 H, $J_{1\text{-}2}$ 7.7 Hz, H-1, β anomer), 4.25 (dd, 1 H, $J_{5\text{-}6}$ 5.1, J_{gem} 10.4 Hz, H-6eq, β anomer) 4.18 (dd, 1 H, $J_{5\text{-}6}$ 5.0, J_{gem} 10.3 Hz, H-6eq, α anomer), 3.99 (ddd, 1 H, $J_{4\text{-}5}\approx J_{5\text{-}6ax}$ 10.1 Hz, H-5, α anomer), 3.84 (dd, 1 H, $J_{2\text{-}3}\approx J_{3\text{-}4}$ 9.2 Hz, H-3, α anomer), 3.77 (dd, 1 H, H-6ax, β anomer), 3.73 (dd, 1-H, H-6ax, α anomer), 3.66-3.58 (m, 4H, H-2, H-4, α anomer, H-3, H-4, β anomer), 3.46 (1 H, ddd, $J_{4\text{-}5}\approx J_{5\text{-}6}$ 9.5 Hz, H-5 β anomer), 3.37 (ddd, 1 H, $J_{2\text{-}3}\approx J_{3\text{-}4}$ 8.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 129.8-128.4, 127.2, 102.6, 102.5, 99.0, 94.9, 83.5, 82.8, 82.5, 80.1, 76.8, 75.8, 75.6, 74.1, 70.4, 69.8, 67.6, 63.6; Anal. Calcd. For $C_{20}H_{22}O_6$; C, 67.03; H, 6.28; O, 26.79; Found; C, 66.82; H, 6.28.

1,2-di-O-acetyl-4-6-O-benzylidene-3-O-benzyl-β-D-glucopyranose (**94**).

4,6-*O*-benzylidene-3-*O*-benzyl-D-glucopyranose (**93**) (3.84 g, 10.6 mmol) was dissolved in a solution of acetic anhydride (50 mL), acetic acid (10 mL) and sodium acetate (3.0 g) was added. The mixture was warmed to reflux over 20 minutes and allowed to cool to room temperature. The solvent was removed under vacuum and the colourless oil was taken up in dichloromethane (200 mL). The solution was washed repeatedly with water, dried over sodium sulfate and concentrated to yield a 6:1 β:α anomeric mixture (4.13g, 88%) from which the β

anomer could be crystallized with EtOAc hexane mixtures; m.p. 162-164 °C $[\alpha]_D$ +16° (*c* 1.3, $CHCl_3$); ^1H NMR (500 MHz, $CHCl_3$) δ 7.48-7.24 (m, 10 H, Ar), 5.68 (d, 1 H, J_{1-2} 8.1 Hz, H-1), 5.56 (s, 1 H, $O_2C\underline{H}Ph$), 5.13-5.10 (2 H, m, H-2, H-3), 4.87 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 4.66 (d, 1 H, $OC\underline{H}_2Ph$), 4.36 (dd, 1 H, J_{4-5} 4.9, J_{5-6} 10.4 Hz, H-6ax), 3.80 (m, 2 H, H-4, H-6eq), 3.57 (ddd, 1 H, $J_{4-5}\approx J_{5-6ax}$ 9.7, J_{5-6eq} 4.9 Hz, H-5), 2.07 (s, 3 H, $COCH_3$), 1.96 (s, 1 H, $COCH_3$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 169.0, 137.9, 136.9, 129.0, 128.3, 128.2, 127.8, 127.7, 126.0, 125.9, 101.3, 92.5, 81.2, 78.4, 74.3, 71.8, 68.4, 66.9, 20.9, 20.8. Anal. Calcd. For $C_{20}H_{22}O_6$; C, 65.15; H, 5.92; O, 28.93; Found; C, 64.97; H, 6.03.

2-O-Acetyl-4,6-O-benzylidene-3-O-benzyl-D-glucopyranose (**95**).

1,2-di-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-benzyl-β-D-glucopyranose (**94**) (4.13g, 93.4 mmol) was dissolved in THF (40 ml) and benzyl amine (1.1mL, 101 mmol) was added. The mixture was stirred for 16 h at room temperature and concentrated to a yellow syrup under vacuum. Chromatography on silica gel using hexane:EtOAc (2:1) yielded a colourless syrup (2.9 g, 78%) which crystallized upon standing. m.p. 149-150 °C; $[\alpha]_D$ +21° (*c* 0.98, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.49-7.24 (10 H, m, Ar), 5.58 (1 H, s, O_2CHPh, α anomer), 5.57 (1 H, s, O_2CHPh, β anomer), 5.42 (d, 1 H, J_{1-2} 3.9 Hz, H-1, α anomer), 4.89-4.84 (m, 3 H, H-2 α anomer, 2($OC\underline{H}_2Ph$)), 4.80 (dd, 1 H, $J_{1-2}\approx J_{2-3}$ 8.9 Hz, H-2 β anomer), 4.68 (d, 1 H, J 11.7, $OC\underline{H}_2Ph$), 4.66 (d, 1 H, J 11.9, $OC\underline{H}_2Ph$), 4.65 (d, 1 H, H-1, β anomer), 4.34 (dd, 1 H, J_{5-6} 5.0 , J_{gem} 10.5 Hz, H-6eq, β anomer), 4.26 (dd, 1 H, J_{5-6}, 5.0, J_{gem} 10.2 Hz, H-6eq α anomer), 4.09 (ddd, $J_{5-6ax}\approx J_{4-5}$ 9.9 Hz, H-5 α anomer), 4.05 (dd, 1 H, $J_{2-3}\approx J_{3-4}$ 9.5 Hz, H-3, α anomer), 3.80-3.68 (m, 4 H, H-6ax, α anomer, H-3, H-4, H-6ax, β anomer), 3.45 (ddd, 1 H, $J_{5-6ax}\approx J_{4-5}$ 10.1 Hz, H-5 β anomer), 2.06 (s, 3 H, $COCH_3$, α anomer), 2.05 (s, 3 H, $COCH_3$, α anomer), ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 137.1, 128.9-125.9, 101.3, 101.3, 96.4, 91.2, 82.1, 81.6, 78.0, 75.8, 75.7, 74.8, 74.5, 73.1, 68.9, 68.6, 66.6, 62.6, 21.0. Anal. Calcd. For $C_{24}H_{24}O_7$; C, 65.99; H, 6.09; O, 27.97; Found; C, 65.79; H, 6.18

2-O-Acetyl-4,6-O-Benzylidene-3-O-benzyl-β-D-glucopyranosyl trichloroacetimidate (**96**).

2-O-Acetyl-4,6-*O*-benzylidene-3-*O*-benzyl-D-glucopyranose (**95**) (1.6 g, 4.0 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. Trichloroacetonitrile (6.0 mmol, 600 μL) was added followed by DBU (20 μL, 0.001 mmol). The ice bath was removed, the reaction warmed to room temperature and stirring was continued for 2 h. Concentration gave a dark brown syrup which was purified by silica gel chromatography to give two products (1.86 g, 86%)

in a 4:1 ratio which were identified as β and α anomers respectively. β anomer $[\alpha]_D$ +28.7 °(*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (1 H, s, NH), 7.49-7.24 (m, 10 H, Ar), 5.85 (d, 1 H, J_{1-2} 7.9 Hz, H-1), 5.58 (s, 1 H, O_2CHPh), 5.29 (dd, 1 H, $J_{1-2}\approx J_{2-3}$ 8.[illegible] Hz, H-2), 4.86 (d, 1 H, J 12.3 Hz, OCH_2Ph) 4.69 (d, 1 H, OCH_2Ph), 4.41 (dd, 1 H, J_{5-6} 5.0, J_{gem} 10.4 Hz, H-6eq), 3.89 (dd, 1 H, $J_{4-5}\approx J_{3-4}$ 9.3 Hz, H-4), 3.82 (1 H, dd, J_{5-6} 10.8 Hz, H-6ax), 3.80 (dd, 1 H, H-3), 3.64 (ddd, 1 H, H-5), 1.95 (3H, s, $COCH_3$), ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 161.1, 129.0-125.9, 101.4, 96.2, 81.1, 81.5, 78.2, 74.0, 71.6, 68.6, 66.9, 20.8; ES HRMS Calcd. for $C_{24}H_{24}NO_7NaCl_3$ 566.0516 found 566.0521;

α anomer $[\alpha]_D$ +37.6 °(*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (s, 1 H, NH), 7.49-7.23 (m, 10 H, Ar), 6.49 (d. 1 H, J_{1-2} 3.7 Hz, H-1), 5.60 (s, 1 H, O_2CHPh), 5.07 (1 H, dd, J_{2-3} 9.7 Hz, H-2), 4.91 (d, 1 H, J 11.7 Hz, OCH_2Ph), 4.73 (d, 1 H, OCH_2Ph), 4.34 (dd, 1 H, J_{5-6} 4.9,, J_{gem} 10.4 Hz, H-6eq), 4.12 (dd, 1 H, J_{3-4} 9.7 Hz, H-3), 4.04 (ddd, 1 H, $H_{4-5}\approx H_{5-6ax}$ 10.1 Hz, H-5), 3.81 (dd, 1 H, H-6eq), 3.77 (1 H. dd, H-4), 1.97 (s, 3 H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 160.9, 138.1, 136.9, 129.0-125.9, 101.4, 93.9, 81.4, 75.6, 74.8, 68.6, 65.2, 20.6; ES HRMS for $C_{24}H_{24}NO_7NaCl_3$ 566.0516 found 566.0520

Allyl (2-O-acetyl-4-6-O-benzylidene-3-O-benzyl-β-D-glucopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**97**).

Glycosyl acceptor (**67**) (1.1g 1.2 mmol) and 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate (**96**) (780 mg, 1.4 mmol) were dissolved in dichloromethane (10 mL) at room temperature. To the stirred solution was added TMSOTf (50 μL, 50μM) and the reaction stirred for 15 minutes at room temperature. Triethylamine (20 μL) was then added and the reaction was concentrated to give yellow oil. Column chromatography in toluene:ethylacetate (8:1)gave a colourless syrup (1.2g, 76%) of the title compound; $[\alpha]_D$ −54.2° (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.00 (40 H, Ar), 5.81 (OCH_2CHCH_2), 5.50 (s, 1 H , O_2CHPh), 5.32 (d, 1 H, J_{1-2} 8.1 Hz, H-1"), 5.20 (dd, 1 H, J_{2-3} 8.3 Hz, H-2"), 5.17 ($OCH_2CHCHaHb$), 5.10 ($OCH_2CHCHaHb$), 4.90 (d, 1 H, J 10.8 Hz, OCH_2Ph), 4.86 (d, 1 H, J 10.6 Hz, OCH_2Ph), 4.78 (d, 1 H, J 11.8 Hz, OCH_2Ph), 4.76 (d, 1 H, J 12.2 Hz, OCH_2Ph), 4.75 (d, 1 H, J 12.2 Hz, OCH_2Ph), 4.67 (s, 1 H, H-1') 4.67-4.61 (m, 3 H, 3(OCH_2Ph)), 4.54-4.43 (m, 5 H, 4(OCH_2Ph), H-2), 4.38-4.31 (m, 4 H, 2(OCH_2Ph), H-6ax", H-1), 4.14 (d, 1 H, J_{2-3} 3.1 Hz, H-2'), 3.97 (OCH_2CHCH_2), 3.90-3.75 (m, 5 H, H-4, H-6a', H-3", H-4", H-6eq"), 3.68-3.57 (m, 5-H, H-6a, H-6b, H4',H-6a' H-5"), 3.49-3.44 (m, 3 H, H-3, H-3', H-5'), 3.30 (ddd, $J_{4-5}\approx J_{5-6}$ 3.5, J_{5-6} 9.9 Hz, H-5), 1.96 (s, 3 H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 138.5, 138.4, 138.2,

138.2, 138.2, 138.0, 137.8, 137.3, 133.9, 129.0-127.5, 126.1, 117.0, 102.3, 101.4, 101.4, 100.3, 81.6, 80.8, 80.6, 79.0, 75.7, 75.6, 75.3, 75.2, 74.6, 74.4, 73.7, 73.3, 73.3, 73.0, 72.6, 70.8, 70.6, 70.1, 70.0, 69.0, 68.9, 66.1, 21.3; ES HRMS Calcd. for $C_{24}H_{24}O_7$ 1327.5601 found 1327.5601

Allyl (4,6-O-benzylidene-3-O-benzyl-β-D-glucopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**98**).

The trisaccharide (**97**) (900 mg, 0.69 mmol) was dissolved in THF (5 mL) and methanol was added (15 mL) followed by a small piece of sodium metal (~10 mg). The reaction was stirred for 16 h and concentrated to dryness. Column chromatography in toluene:EtOAc (8:1) gave a colourless syrup (823 mg, 94%) containing the title compound (**98**); $[\alpha]_D$ −56.9° (*c* 1.5, $CHCl_3$) ^{1}NMR (500 MHz, $CDCl_3$) δ 7.43-6.99 (40 H, Ar), 5.83 ($OCH_2C\underline{H}CH_2$), 5.50 (s, 1 H , $O_2C\underline{H}Ph$), 5.19 ($OCH_2CHCHa\underline{Hb}$), 5.14 ($OCH_2CHC\underline{Ha}Hb$), 4.98 (d, 1 H, J 11.8 Hz, $OC\underline{H}_2Ph$), 4.97 (d, 1 H, J 10.9 Hz, $OC\underline{H}_2Ph$), 4.93 (d, 1 H, J 10.9 Hz, $OC\underline{H}_2Ph$), 4.93 (s, 1 H, H-1'), 4.83 (d, 1 H, J 12.2 Hz, $OC\underline{H}_2Ph$), 4.81 (d, 1 H, J 6.9 Hz, H-1"), 4.76 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.72 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.63 (d, 1 H, J 12.2 Hz, $OC\underline{H}_2Ph$), 4.53 (d, 1 H, J 12.8 Hz, $OC\underline{H}_2Ph$), 4.50 (d, 1 H, J 13.1 Hz, $OC\underline{H}_2Ph$), 4.48 (d, 1 H, J 11.2 Hz, $OC\underline{H}_2Ph$), 4.45-4.35 (7 H , H-2', H-1, 4($OC\underline{H}_2Ph$), $OC\underline{H}_2CHCH_2$), 4.16 (1 H, m, H-4"), 4.10 (1 H, dd, $J_{3-4}\approx J_{4-5}$ 9.5 Hz, H-4'), 4.00 ($OC\underline{H}_2CHCH_2$), 3.81-3.60 (10 H, m, H-3, H-5, H-6a, H-6b, H-6a', H-6b', H-2", H-3", H-6a'', H-6b''), 3.55 (1 H, dd, J_{1-2} 2.9, J_{2-3} 9.6 Hz, H-2), 3.50-3.45 (2 H, H-4, H-3'), 3.39 (1 H, ddd, $J_{4-5}\approx J_{5-6ax}$ 9.8, J_{4-5eq} 5.0, H-5"), 3.34 (1 H, ddd, J_{4-5} 9.9, J_{5-6a} 3.1, J_{5-6b} 4.6 Hz, H-5') ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.4, 138.9, 138.5, 138.3, 138.0, 137.9, 137.9, 137.9, 137.5, 133.6, 129.0, 127.0, 125.2, 117.3, 105.9, 101.2, 100.1, 99.8, 82.1, 82.1, 80.9, 80.1, 80.4, 75.5, 75.4, 75.3, 74.8, 74.5, 73.8, 73.6, 73.4, 71.3, 70.8, 70.3, 70.1, 70.1, 70.1, 69.3, 69.3, 68.6, 66.4; ES HRMS $C_{77}H_{82}NaO_{16}$ 1285.5495 found 1285.5495

Allyl (4,6-O-benzylidene-3-O-benzyl-2-O-trifluoromethylsulfonyl-β-D-glucopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**99**).

The alcohol (**98**) (800mg, 0.633 mmol) was dissolved in dichloromethane (5 mL) and pyridine (300 μL) was added followed by *N,N*-dimethyl-4-aminopyridine (50 mg, 0.40 mmol). The solution was cooled to 0 °C and trifluoromethyanesulfonic anhydride (200 μL, 1.18 mmol) was added dropwise, the reaction was then warmed to room temperature and stirred for 16 h. The solution was diluted with dichloromethane, washed with a bicarbonate solution, dried over sodium sulfate and concentrated to a brown oil. Column chromatography in toluene:EtOAc (8:1)

gave a colourless syrup (789 mg, 89%). $[\alpha]_D$ –72.9 °(*c* 1.0, $CHCl_3$) ^{1}NMR (500 MHz, $CDCl_3$) δ 7.39-6.96 (40 H, Ar), 5.82 ($OCH_2C\underline{H}CH_2$), 5.75 (d, 1 H, J_{1-2} 8.0 Hz, H-1"), 5.44 (s, 1 H, $O_2C\underline{H}Ph$), 5.20 ($OCH_2CHCHa\underline{Hb}$), 5.12 ($OCH_2CHC\underline{Ha}Hb$), 4.97 (d, 1 H, J 10.7 Hz, $OC\underline{H}_2Ph$), 4.87 (d, 1 H, J 10.9 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1 H, J 11.6 Hz, $OC\underline{H}_2Ph$), 4.75 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.70 (d, 1 H, J 12.4 Hz, $OC\underline{H}_2Ph$), 4.68 (d, 1 H, J_{2-3} 2.9 Hz, H-2'), 4.60-4.33 (m, 14 H, 9($OC\underline{H}_2Ph$), H-2", H-6a", H-1, H-2, $OC\underline{H}_2CHCH_2$), 3.96 (dddd, 1 H, $OC\underline{H}_2CHCH_2$), 3.87-3.74 (m, H-4, H-6a, H-4' , H6a', H-6b'), 3.70- 3.59 (m, 5 H, H-6a, H-5', H-4", H-5", H-6b"), 3.57 (dd, 1 H, J_{3-4} 9.2 Hz, H-3'), 3.52 (dd, 1 H, J_{2-3} 3.4, J_{3-4} 9.3 Hz, H-3), 3.42 (dd, 1 H, J_{2-3}≈J_{3-4} 9.0 Hz, H-3"), 3.31 (ddd, 1 H, J_{4-5} 8.1, J_{5-6a} 3.6 , J_{5-6b} 1.6 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5-136.9, 133.6, 128.9-127.3, 125.2, 117.4, 101.4, 101.0, 100.5, 97.4, 85.4, 82.3, 81.1, 80.5, 77.9, 76.3, 75.5, 75.3, 75.2, 74.9, 74.7, 74.0, 73.7, 73.3, 70.7, 70.7, 70.4, 70.0, 69.6, 69.6, 68.5, 68.3, 66.2 EMS Calcd. for $C_{78}H_{81}F_3O_{18}S$. 1417.5 found 1417.5

Allyl (4,6-O-benzylidene-3-O-benzyl-2-deoxy-2-thioacetyl-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**100**).

The triflate (**99**) (600mg, 0.43 mmol) was dissolved in DMF (5 ml) and potassium thioacetate was added (3 g). The flask was purged with argon and immersed in an oil bath at 150 °C for 5 min. The bath was removed and reaction was cooled to room temperature. The dark brown solution was and diluted with toluene (50 mL) and repeatly washed with water to give a yellow solution. After drying over sodium sulfate and concentration the yellow oil was subjected to column chromatography in toluene in a gradient to 10 % EtOAc gave a colourless syrup (359 mg, 63%); $[\alpha]_D$ –65.2° (c 0.7, $CHCl_3$) ^{1}NMR (500 MHz, $CDCl_3$) δ 7.50-6.90 (40 H, m, Ar), 5.84 (s, 1 H, H-1"), 5.83 ($OCH_2C\underline{H}CH_2$), 5.48 (s, 1 H , O_2CHPh), 5.19 ($OCH_2CHCHa\underline{Hb}$), 5.14 (s, 1 H, H-1'), 5.12 ($OCH_2CHC\underline{Ha}Hb$), 4.92 (d, 1 H, J 10.7 Hz, $OC\underline{H}_2Ph$), 4.84 (d, 2 H, J 10.5 Hz, 2 ($OC\underline{H}_2Ph$)), 4.85 (d, 1 H, J 11.3 Hz, $OC\underline{H}_2Ph$), 4.72 (d, 1 H, J_{2-3} 2.1 Hz, H-2'), 4.68 (d, 1 H, J 11.3 Hz, $OC\underline{H}_2Ph$), 4.63-4.62 (m, 2 H, H-2, H-2"), 4.54-4.36 (m, 10 H, 7($OC\underline{H}_2Ph$), $OC\underline{H}_2CHCH_2$, H-6ax", H-1), 4.31 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.68 (d, 1 H, J 12.1, $OC\underline{H}_2Ph$), 3.96 ($OC\underline{H}_2CHCH_2$), 3.83-3.52 (m, 13 H, H-3, H-4, H-6a, H-6b, H-3', H-4', H-5', H-6a', H-6b', H-3", H-4", H5", H-6eq"), 3.31 (ddd, 1 H, J_{4-5} 3.7, J_{5-6} 1.7, J_{5-6} 11.5 Hz, H-5), 2.21 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6-135.4, 133.5, 128.9-127.1, 117.3, 101.5, 101.0, 100.3, 97.2, 81.0, 80.5, 80.3, 76.3, 75.8, 75.6, 75.3, 75.2, 75.2, 74.2, 73.8, 73.4, 71.4, 70.7, 70.5, 70.0, 69.5, 69.3, 68.6, 68.6, 68.4, 67.6, 30.7; ES HRMS Calcd. for $C_{79}H_{84}O_{16}SNa$ 1343.5372 found 1343.5370

Allyl (4,6-O-benzylidene-3-O-benzyl-2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**101**).

Thioacetate (**100**) (300mg, 0.23 mmol) was dissolved in THF (5 mL), cyclohexene (1 mL), ethanol (1 ml) and hydrazine hydrate (100μL, ~1.7 mmol) were added. The solution was stirred at room temperature for 3 h and diluted with toluene. Concentration gave a colourless syrup which could be chromatographed in toluene:ethyl actetate(8:1) to yield the thiol **101** (262 mg, 89%); $[\alpha]_D$ −78.1° (*c* 1.1, $CHCl_3$) ^{1}NMR (600 MHz, $CDCl_3$) δ 7.47-6.90 (40 H, Ar), 5.87 (OCH_2CHCH_2), 5.57 (s, 1 H, O_2CHPh), 5.51 (d, 1 H, J_{1-2} 1.8 Hz, H-1"), 5.22 ($OCH_2CHCHaHb$), 5.12 ($OCH_2CHCHaHb$), 5.14 (s, 1 H, H-1'), 4.95 (d, 1 H, J 10.8 Hz, OCH_2Ph), 4.94 (d, 1 H, J 10.2 Hz, OCH_2Ph), 4.90 (d, 1 H, J 11.5 Hz, OCH_2Ph), 4.76 (d, 1 H, J 10.3 Hz, OCH_2Ph), 4.72 (d, 1 H, J 11.7 Hz, OCH_2Ph), 4.71 (d, 1 H, J_{2-3} 2.4 Hz, H-2'), 4.61 (d, 1 H, J_{2-3} 3.5 Hz, H-2), 4.58 (d, 1 H, J 10.8 Hz, OCH_2Ph), 4.53 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.50-4.40 (m, 5 H, 3(OCH_2Ph), OCH_2CHCH_2, H-1), 4.33 (d, 1 H, J 10.1 Hz, OCH_2Ph), 4.29 (dd, J_{5-6} 4.9, J_{gem} 10.3 Hz, H-6eq"), 4.25 (dd, $J_{5-6} \approx J_{gem}$ 9.4 Hz, H-6ax"), 4.24 (d, 1 H, J 10.1 Hz, OCH_2Ph), 4.19 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.11 (d, 1 H, J 11.9 Hz, OCH_2Ph), 4.10 (ddd, J_{2-3} 4.5, J_{2-SH} 2.6 Hz, H-2"), 4.01 (OCH_2CHCH_2), 3.93 (dd, 1 H, $J_{3-4} \approx J_{4-5}$ 9.3 Hz, H-4'), 3.85 (dd, $J_{3-4} \approx J_{4-5}$ 10.3 Hz, H-4"), 3.81 (dd, J_{5-6} 1.5, J_{gem} 10.4 Hz, H-6a'), 3.76-3.67 (m, 5 H, H-4, H-6a, H-6b, H-6b', H-3"), 3.62 (dd, 1 H, H-3'), 3.59 (dd, 1 H, J_{3-4} 9.3 Hz, H-3), 3.54 (ddd, 1H, J_{5-6} 6.0 Hz, H-5'), 3.50 (ddd, 1 H, H-5"), 3.38 (ddd, 1 H, J_{5-6a} 1.8, J_{5-6b} 4.2, J_{4-5} 9.7 Hz, H-5), 2.27 (d, 1H, SH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 137.5, 133.5, 129.0-127.1, 117.4, 101.5, 100.7, 100.1, 99.1, 80.7, 80.6, 78.0, 76.8, 75.5, 75.4, 75.2, 75.2, 74.5, 74.4, 73.7, 73.4, 71.0, 70.6, 70.4, 70.2, 70.1, 69.8, 69.7, 68.7, 68.5, 67.7; ES HRMS Calcd. for $C_{77}H_{82}O_{15}SNa$ 1301.5267 found 1301.5293

Allyl (3,4,6-tri-O-p-chlorobenzyl-1-thio-β-D-mannopyranosyl)(1→2)(4,6-O-benzylidene-3-O-benzyl-2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside (**102**).

Thiol (**101**) (250 mg, 0.200 mmol) and freshly prepared 3,4,6 tri-*O*-*p*-chlorobenzyl-α-D-*arabino*-hexopyranos-2-ulosyl bromide (240 mg, 0.390 mmol) were dissolved in dichloromethane (2 mL) at room temperature. The flask was purged with argon and lutidine (55 μL, 0.470 mmol) was added. The reaction was stirred at room temperature for 2 h and concentrated to dryness. The syrup was dissolved in THF (10 ml) and cooled to −78 °C. L-Selectride (1.0M, 1.5 mL) was then added dropwise and the cooling bath was removed. Once the

reaction had reached room temperature the solution was quenched with methanol (2 mL) and diluted with dichloromethane. Washing with water, bicarbonate solution, and finally a brine solution gave a pale yellow solution. Drying over sodium sulfate, and concentration followed by silica gel chromatography gave a colourless oil (180 mg, 49%) and similarly the α anomer (45 mg) $[\alpha]_D$ –79.4° (*c* 0.81, $CHCl_3$) ^{1}NMR (600 MHz, $CDCl_3$) δ 7.52-6.85 (52 H, Ar), 5.91 (s, 1 H, H-1"), 5.82 (dddd, $OCH_2C\underline{H}CH_2$), 5.52 (s, 1 H , $O_2C\underline{H}Ph$), 5.52 (s, 1 H, H-1'"), 5.22 (s, 1 H, H-1'), 5.19 ($OCH_2CHCHa\underline{Hb}$), 5.13 ($OCH_2CHC\underline{Ha}Hb$), 4.94 (d, 1 H, J 10.1 Hz, $OC\underline{H}_2Ph$), 4.93 (d, 1 H, J_{2-3} 3.1 Hz, H-2'), 4.90 (d, 1 H, J 10.4 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1 H, J 11.0 Hz, $OC\underline{H}_2Ph$), 4.77 (d, 1 H, J 10.9 Hz, $OC\underline{H}_2Ph$), 4.71 (d, 1 H, J 12.3 Hz, $OC\underline{H}_2Ph$), 4.63 (d, 1 H, J 10.9 Hz, $OC\underline{H}_2Ph$), 4.62 (d, 1 H, J 12.8 Hz, $OC\underline{H}_2Ph$), 4.60 (d, 1 H, J_{2-3} 4.0 Hz, H-2), 4.55 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.51 (dd, J_{5-6} 5.0, J_{gem} 10.6 Hz, H-6ax"), 4.49-4.37 (m, 10 H, 8($OC\underline{H}_2Ph$), H-1, $OC\underline{H}_2CHCH_2$), 4.32 (d, 1 H, J 12.4 Hz, $OC\underline{H}_2Ph$), 4.07 (d, 1 H, J 11.5 Hz, $OC\underline{H}_2Ph$), 4.04 (d, 1 H, J 12.3 Hz, $OC\underline{H}_2Ph$), 4.03 (d, 1 H, J_{2-3} 4.4 Hz, H-2'"), 3.96 ($OC\underline{H}_2CHCH_2$), 3.93 (d, 1 H, J_{2-3} 4.6 Hz, H-2"), 3.91-3.84 (m, 3 H, H-4, H6a", H6a'), 3.82-3.79 (m, 4 H, H-6a, H-4'", H-6b', $OC\underline{H}_2Ph$), 3.75-3.58 (m, 9 H, H-5'", H-6a'", H-6b'", H-5", H-4", H-4', H-6b), 3.65 (2 H, m, H-3', H-3") 3.56 (dd, J_{3-4} 9.5 Hz, H-3), 3.51 (ddd, J_{4-5} 10.1, J_{5-6} 4.9, J_{5-6} 1.8 Hz, H-5'), 3.48 (dd, J_{3-4} 9.2 Hz, H-3'"), 3.32 (ddd, 1 H, J_{4-5} 9.5, J_{5-6} 3.9, J_{5-6} 1.5 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2-136.3, 133.4, 129.0, 129.1-126.2, 117.4, 101.6 ($^1J_{C1-H1}$ 154.0 Hz, C-1), 101.1, 101.1 ($^1J_{C1-H1}$ 160.9, C-1'), 99.3 ($^1J_{C1-H1}$ 165.6, C-1"), 83.9 ($^1J_{C1-H1}$ 160.4, C-1'"), 81.3, 80.3, 79.6, 79.3, 76.3, 75.8, 75.7, 75.6, 75.4, 75.4, 74.2, 74.1, 73.5, 72.9, 70.5, 70.1, 70.0, 70.0, 69.8, 69.7, 69.6, 69.5, 69.4, 68.5, 68.4, 68.4, 68.3, 68.3, 68.1, 68.1, 68.0; EMS Calcd. for $C_{107}H_{113}Cl_3O_{17}S$ 1837.6 found 1837.6 correct isotopic intensity pattern

***3-(2-Aminoethylthio)-propyl (3,4,6-tri-O-p-chlorobenzyl-1-thio-β-D-mannopyranosyl)(1→2)(4,6-O-benzylidene-3-O-benzyl-2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-O-benzyl-β-D-mannopyranoside* (103).**

Allyl glycoside (**102**) (50 mg, 0.028 mmol) was added to a quartz vessel and dissolved in a minimum amount of dichloromethane (400 μL). Methanol (5 mL) was then added followed by 2-aminoethanethiol hydrochloride (1.0 g) and irradiation (365 nm) was carried out for 13 h. The solution was diluted with dichloromethane and washed with 1M sodium hydroxide. The combined organic layer was dried, concentrated and subjected to column chromatography in dichloromethane containing 2% methanol to give a colourless syrup (38 mg, 73%); $[\alpha]_D$ –70.2° (*c* 0.5, $CHCl_3$); ^{1}NMR (600 MHz, $CDCl_3$) δ 7.48-6.82 (52 H, Ar), 5.88 (s, 1 H, H-1"), 5.51 (s, 1 H,

$O_2C\underline{H}Ph$), 5.50 (s, 1 H, H-1'''), 5.13 (s, 1 H, H-1'), 4.90 (d, 1 H, J 10.3 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1 H, J 10.3 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1 H, $J_{2\text{-}3}$ 3.1 Hz, H-2'), 4.82 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.67 (d, 1 H, J 12.5 Hz, $OC\underline{H}_2Ph$), 4.58 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.58 (d, 1 H, J 12.6 Hz, $OC\underline{H}_2Ph$), 4.57 (d, 1 H, $J_{2\text{-}3}$ 3.8 Hz, H-2), 4.52 (d, 1 H, J 11.7 Hz, $OC\underline{H}_2Ph$), 4.48-4.38 (m, 9 H, 7($OC\underline{H}_2Ph$), H-1, H-6a''), 4.33 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.30 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.04 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.02 (d, 1 H, J 11.4 Hz, $OC\underline{H}_2Ph$), 4.02 (d, 1 H, J 3.3 Hz, H-2'''), 3.95 (dt, 1 H, J_{gem} 9.7, J_{vic} 6.2 Hz, $OC\underline{H}_2CH_2$), 3.89 (d, 1 H, J 4.8 Hz, H-2''), 3.87-3.58 (m, 18 H, H-3, H-4, H-6a, H-6b, H-3', H-4', H-6a', H-6b', H-4'', H-5'', H-6b'', H-4''', H-5''', H-6a''', H-6b''', $OC\underline{H}_2Ph$), 3.55 (dd, $J_{3\text{-}4}$ 9.0 Hz, H-3''), 3.48-3.43 (m, 3 H, H-5', H-3''', $OC\underline{H}_2CH_2$), 3.32 (ddd, 1 H, $J_{4\text{-}5}$ 9.3, $J_{5\text{-}6}$ 1.8, $J_{5\text{-}6}$ 4.0 Hz, H-5), 3.03 (t, 2 H, J 7.0 Hz, $CH_2C\underline{H}_2NH_2$), 2.70 (ABX_2, 2 H, SCH_2CH_2), 2.50 (t, 2 H, J 6.7 Hz, $OCH_2CH_2C\underline{H}_2S$), 1.79 (p, 2 H, J 6.7 Hz, $OCH_2C\underline{H}_2CH_2S$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.1($^2J_{C1\text{-}H1}$ 167.3 Hz, H-1''), 103.8 ($^2J_{C1\text{-}H1}$ 158.3 Hz, H-1'''), 100.9 ($^2J_{C1\text{-}H1}$ 158.3 Hz, H-1'), 101.9 ($^2J_{C1\text{-}H1}$ 152.7 Hz, H-1); EMS Calcd. for $C_{109}H_{120}Cl_3O_{17}S_2$ 1914.5 found 1914.6 correct isotope pattern

*Propyl (3,4,6-tri-*O*-p-chlorobenzyl-1-thio-β-D-mannopyranosyl)(1→2)(4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside* (**104**).

Allyl glycoside (50mg, 0.028 mmol) was dissolved in THF (5 mL), ethanol (1 mL) and hydrazine hydrate (500 μL). The reaction was stirred open to the atmosphere for 3 h then diluted with toluene. Concentration followed by column chromatography in toluene:EtOAc (8:1) gave the propyl glycoside as a colorless syrup (45 mg, 90%). $[\alpha]_D$ -68.5° (*c* 1.2, $CHCl_3$) ^{1}NMR (600 MHz, $CDCl_3$) δ 7.49-6.80 (52 H, Ar), 5.86 (d, H-1, $J_{1\text{-}2}$ 0.9 Hz, H-1''), 5.49 (s, 1 H, $O_2C\underline{H}Ph$), 5.48 (s, 1 H, H-1'''), 5.17 (s, 1 H, H-1'), 4.90 (d, 1 H, J 9.9 Hz, $OC\underline{H}_2Ph$), 4.90 (d, 1 H, $J_{2\text{-}3}$ 3.5 Hz, H-2'), 4.87 (d, 1 H, J 10.4 Hz, $OC\underline{H}_2Ph$), 4.83 (d, 1 H, J 10.8 Hz, $OC\underline{H}_2Ph$), 4.74 (d, 1 H, J 11.2 Hz, $OC\underline{H}_2Ph$), 4.67 (d, 1 H, J 12.4 Hz, $OC\underline{H}_2Ph$), 4.59 (d, 1 H, J 11.3 Hz, $OC\underline{H}_2Ph$), 4.59 (d, 1 H, J 12.5 Hz, $OC\underline{H}_2Ph$), 4.53 (d, 1 H, $J_{2\text{-}3}$ 3.7 Hz, H-2), 4.53 (d, 1 H, J 11.2 Hz, $OC\underline{H}_2Ph$), 4.48-4.39 (m, 8 H, 7($OC\underline{H}_2Ph$), H-6a''), 4.36 (d, 1 H, J 11 Hz, $OC\underline{H}_2Ph$), 4.33 (s, 1 H, H-1), 4.29 (d, 1 H, J 12.2 Hz, $OC\underline{H}_2Ph$), 4.04 (d, 1 H, J 11.6 Hz, $OC\underline{H}_2Ph$), 4.02 (d, 1 H, J 11.8 Hz, $OC\underline{H}_2Ph$), 4.00 (d, 1 H, J 2.9 Hz, H-2'''), 3.91 (d, 1 H, J 4.8 Hz, H-2''), 3.89-3.58 (m, 18 H, H-3, H-4, H-6a, H-6b, H-4', H-6a', H-6b', H-4'', H-5'', H-6b'', H-4''', H-5''', H-6a''', H-6b''', $OC\underline{H}_2Ph$, $OC\underline{H}_2CH_2$), 3.56 (dd, $J_{3\text{-}4}$ 9.5 Hz, H-3''), 3.53 (dd, $J_{3\text{-}4}$ 9.5 Hz, H-3'), 3.46 (ddd, $J_{4\text{-}5}$ 9.3, $J_{5\text{-}6}$ 4.6, $J_{5\text{-}6}$ 1.7 Hz, H-5'), 3.45 (dd, $J_{3\text{-}4}$ 7.5 Hz, H-3'''), 3.30 (ddd, $J_{4\text{-}5}$ 9.9, $J_{5\text{-}6}$ 4.13, $J_{5\text{-}6}$ 1.8 Hz, H-5) 3.27 (dt, J_{gem} 9.3, J_{vic}

6.9 Hz, OCH_2CH_2), 1.53 (hextet, 2 H, J 7.0 Hz, $OCH_2CH_2CH_3$), 0.85 (t, 2 H, J 7.0 Hz, $OCH_2CH_2CH_3$); NMR (125 MHz, $CDCl_3$) δ 138.2-136.3, 133.1, 133.0, 128.9-127.2, 102.0, 101.6, 101.2, 99.3, 83.9, 81.3, 80.3, 79.6, 79.3, 76.2, 75.9, 75.7, 75.6, 75.4, 75.4, 74.2, 74.2, 74.2, 73.8, 73.5, 72.8, 72.0, 70.2, 70.1, 70.0, 69.8, 69.7, 69.7, 69.6, 69.4, 68.6, 68.4, 68.4, 68.1, 68.0, 48.8, 22.9, 10.7; EMS Calcd. for $C_{107}H_{115}C_{13}O_{17}S$ 1814.6 found 1814.6

Propyl (1-thio-β-D-mannopyranosyl)(1→2)(2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside (**105**).

Into freshly distilled ammonia (75 mL) containing sodium metal (100 mg) at –78 °C was added a solution of the propyl glycoside (**104**) (30 mg, 0.016 mmol) in THF (3 mL) and *t*-butanol (1 mL). The reaction was stirred at –78 °C for 20 min then quenched with methanol. Ammonia was allowed to evaporate under a stream of argon and the remaining solvent was removed under vacuum. The resulting solid was taken up in water (20 mL) and filtered through a 0.2 μM membrane. It was then brought to neutral pH with the addition of 5 M acetic acid and concentrated to a solid under vacuum. Purification on reverse phase silica (C18) was accomplished with a water methanol gradient to yield a colourless glass (10 mg 84%); ^{1}H NMR (800 Hz D_2O) δ 5.17 (1 H, s, H-1"), 5.00 (1 H, s, H-1'"), 4.88 (1 H, s, H-1'), 4.73 (1 H, s, H-1), 4.39 (1 H, d, J_{2-3} 3.4 Hz, H-2'), 4.24 (1 H, d, J_{2-3} 3.7 Hz, H-2), 4.10 (1 H, d, J_{2-3} 3.4 Hz, H-2'"), 3.95 (1 H, dd, J_{gem} 12.2, J_{vic} 2.2 Hz, H-6a'), 3.93 (1 H, dd, J_{gem} 11.0, J_{vic} 2.2 Hz, H-6a), 3.92 (1 H, dd, J_{gem} 9.2, J_{vic} 2.2 Hz, H-6a'"), 3.91 (1H, dd, J_{3-4} 9.8 hz, H-3"), 3.87 (1 H, dd, J_{gem} 12.6, J_{vic} 2.2 Hz, H-6a"), 3.86 (1 H, dt, J_{vic} 6.6, J_{gem} 9.5 Hz, OCH_2CH_2), 3.80 (1 H, d, J_{2-3} 4.6 Hz, H-2"), 3.72 (1 H, dd, J_{5-6} 6.1 Hz, H-6b), 3.71 (1 H, dd, J_{5-6} 7.1 Hz, H-6b'"), 3.70 (1 H, dd, J_{5-6} 6.5 Hz, H-6b"), 3.68 (1 H, dd, J_{5-6} 6.4 Hz, H-6b'), 3.67 (1 H, dd, J_{3-4} 9.4 Hz, H-3), 3.67 (1 H, dd, J_{3-4} 9.4 Hz, H-3'), 3.62 (1 H, dd, J_{3-4} 9.5 Hz, H-3'"), 3.61 (1 H, dt, J_{vic} 7.1 Hz, OCH_2CH_2), 3.56 (1 H, dd, J_{4-5} 9.5 Hz, H-4'"), 3.47 (1 H, dd, J_{4-5} 9.8 Hz, H-4), 3.45 (1 H, dd, J_{4-5} 9.7 Hz, H-4'), 3.44 (1 H, ddd, H-5'"), 3.39 (1 H, ddd, H-5'), 3.37 (1 H, ddd, H-5"), 3.36 (1 H, ddd, H-5), 3.25 (1 H, dd, J_{4-5} 9.5 Hz, H-4"), 1.62 (2 H, p, J 7.0 Hz, $OCH_2CH_2CH_3$), 0.93 (3 H, t, $CH_2CH_2CH_3$); ^{13}C NMR (125 Hz, $CDCl_3$), 102.2 (J^1_{C-H} 162.4 Hz, C-1'), 102.0 (J^1_{C-H} 163.4 Hz, C-1"), 101.8 (J^1_{C-H} 159.5 Hz, C-1), 86.7 (J^1_{C-H} 157.6 Hz, C-1'"), 80.9 (C-5'"), 80.3 (C-2), 79.4 (C-2'), 77.6, 77.1, 77.1(C-5, C-5', C-5"), 74.7 (C-3'"), 72.9-72.6 (C-3, C-3',C-3"), 72.6 (OCH_2CH_2), 68.7, 68.6, 68.4 (C-4, C-4', C-4"), 67.6 (C-4'"), 62.2-61.7 (C-6, C-6', C-6", C-6'"), 54.3 (C-2"), 23.3 ($CH_2CH_2CH_3$), 10.8 (CH_2CH_3); ES HRMS Calcd. for $C_{27}H_{48}NaO_{20}S$ 747.2357 found 747.2352

3-(2-Aminoethylthio)-propyl (1-thio-β-D-mannopyranosyl)(1→2)(2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside **(106).**

Into freshly distilled ammonia (75 mL) containing sodium metal (100 mg) at –78 °C was added a solution of the tetrasaccharide (**103**) (40 mg, 0.021 mmol) in THF (3 mL) and *t*-butanol (1 mL). The reaction was stirred at –78 °C for 20 min then quenched with methanol. Ammonia was allowed to evaporate under a stream of argon and the remaining solvent was removed under vacuum. The resulting solid was taken up in distilled water (20 mL) and filtered through a 0.2 μM membrane. It was then brought to neutral pH with the addition of acetic acid (5M) and concentrated to a solid under vacuum. Purification on reverse phase silica (C18) was accomplished with a water methanol gradient to yield a colourless glass (12 mg 79%); ^{1}NMR (600 MHz, $CDCl_3$) δ 5.18 (s, H-1, H-1"), 5.02 (s, 1 H, H-1'"), 4.89 (s, 1 H, H-1'), 4.75 (s, 1 H, H-1), 4.39 (d, 1 H, J_{1-2} 3.5 Hz, H-2'), 4.26 (d, 1 H, J_{1-2} 3.1 Hz, H-2), 4.11 (d, 1 H, J_{1-2} 3.3 Hz, H-2'"), 4.01 (dt, J_{gem} 10.1, J_{vic} 6.1 Hz, $OC\underline{H}_2CH_2CH_3$), 3.97 (dd, 1 H, J_{gem} 12.1, J_{5-6} 2.2 Hz, H-6a'), 3.94 (dd, 1 H, J_{gem} 12.1, J_{5-6} 2.2 Hz, H-6a), 3.93 (dd, 1 H, J_{gem} 9.7, J_{5-6} 1.6 Hz, H-6a'"), 3.91 (dd, J_{3-4} 9.5 Hz, H-3"), 3.89 (dd, 1 H, J_{gem} 12.1 Hz, J_{5-6} 2.0 Hz, H-6a"), 3.82 (d, 1 H, J_{1-2} 4.4 Hz, H-2"), 3.77-3.67 (m, 6 H, H-6b, H-6b', H-6b", H-6b'", H-3, OCH_2CH_2), 3.66 (dd, 1 H, J_{3-4} 9.9 Hz, H-3'), 3.63 (dd, 1 H, J_{3-4} 9.5 Hz, H-3'"), 3.58 (dd, J_{4-5} 9.7 Hz, H-4'"), 3.48 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.7 Hz, H-4), 3.47 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.7 Hz, H-4'), 3.45 (ddd, 1 H, H-5'"), 3.40-3.36 (m, 3 H, H-5', H-5", H-5'"), 3.27 (dd, 1 H, $J_{3-4}\approx J_{4-5}$ 9.7 Hz, H-4"), 3.16 (t, 2 H, J 6.6 Hz, $C\underline{H}_2NH_3^+$), 2.84 (t, J 6.8 Hz, $SC\underline{H}_2CH_2NH_3^+$), 2.70-2.68 (m, 2 H, $OCH_2CH_2C\underline{H}_2S$), 1.95 (p, 2 H, J 6.6 Hz, $OCH_2C\underline{H}_2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 102.2 ($^2J_{C1\text{-}H1}$ 163.9 Hz, C-1"), 102.0 ($^2J_{C1\text{-}H1}$ 157.5 Hz, C-1'), 100.9 ($^2J_{C1\text{-}H1}$ 159.6 Hz, C-1), 86.7 ($^2J_{C1\text{-}H1}$ 157.5 Hz, C-1'"), 80.9, 80.1, 79.4, 77.6, 77.1, 74.7, 73.0, 72.8, 72.8, 69.3, 68.7, 68.6, 68.4, 67.6, 62.2, 61.8, 54.3, 39.4, 29.9, 29.6, 28.6; ES HRMS Calcd. for $C_{29}H_{54}NO_{20}S2$ 800.2680 found 800.2676

1-[3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside]-2-ethoxycyclobutene-3,4-dione **(107)**

Amine **84** (7 mg) was dissolved in a 1:1 solution of ethanol:water (2 mL) and 3,4-diethoxy-3-cyclobutene-1,2-dione (8 μL, 5 eq) was added. A saturated solution of sodium carbonate was then added in 2 μL aliquots, allowing the reaction to stir for 5 min between additions. The addition was continued until TLC analysis indicated the free amine had been completely converted into a faster moving product. The reaction was then concentrated and purified by HPLC on C18 silica gel to give 107 (6 mg 74 %); ^{1}H NMR (600 MHz, D_2O) δ 4.85,

4.83 (s, 1 H, H-1'), 4.77, 4.73 (q, 2 H, OCH_2CH_3), 4.72 (s, 1 H, H-1), 4.25 (br s, 1 H, H-2), 4.12 (br, s, 1 H, H-2'), 3.99 (dt, 1 H, J_{gem} 9.2 Hz, J_{vic} 7.1, OCH_2CH_2), 3.91-3.94 (m, 2 H, H-6a, H-6a'), 3.83 (t, J_{vic} 6.81 Hz, SCH_2CH_2NH), 3.77-3.72 (m, 4 H, H6b, H6b', OCH_2CH_2, SCH_2CH_2NH), 3.65-3.55 (m, 4 H, H-3, H-3', H-4, H4'), 3.40-3.33 (2 H, m, H-5, H-5') 2.82 (t, 2 H, J_{vic} 6.4 Hz, SCH_2CH_2NH), 2.69 (t, 2 H, J 6.6 Hz, $CH_2CH_2CH_2S$), 1.94-1.89 (m, 2 H, $OCH_2CH_2CH_2S$); 1.47, 1,44 (t, J 6.8 Hz, OCH_2CH_3); ES HRMS Calcd. for $C_{23}H_{37}NO_{14}SNa$ 606.1832 Found 606.1823

1-[3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside]-2-ethoxycyclobutene-3,4-dione (**108**)

The foregoing amine **85** (14 mg) was treated with diethyl squarate as described for the preparation of **107** to give **108** (13 mg, 78%); ^{1}H NMR (600 MHz, D_2O) δ 4.95 (s, 1 H, H-1"), 4.93, 4.90 (s, 1 H, H-1'), 4.77, 4.73 (q, 2 H, OCH_2CH_3), 4.71 (s, 1 H, H-1), 4.34 (br s, 1 H, H-2'), 4.22 (br s, 1 H, H-2), 4.15 (d, 1 H, $J_{2,3}$ 3.1 Hz, H-2"), 3.99 (dt, 1 H, J_{gem} 10.1 Hz, J_{vic} 5.6, OCH_2CH_2), 3.94-3.91 (m, 3 H, H-6a, H6a', H6a"), 3.83 (t, J_{vic} 6.4 Hz, SCH_2CH_2NH), 3.77-3.71 (m, 5 H, H-6b, H-6b', H6b", OCH_2CH_2, SCH_2CH_2NH), 3.67 (dd, 1 H, $J_{2,3}$ 3.3 $J_{3,4}$ 9.9 Hz, H-3), 3.64-3.56 (m, 4 H, H-3', H-3", H-4', H4"), 3.49 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.7 Hz, H-4), 3.38-3.34 (m, 3 H, H-5, H-5", H-5"), 2.83 (t, 2 H, J_{vic} 6.4 Hz, SCH_2CH_2NH), 2.70 (m, 2 H, $CH_2CH_2CH_2S$), 1.94-1.90 (m, 2H, $OCH_2CH_2CH_2S$), 1.47, 1.45 (t, J 7.1 Hz, OCH_2CH_3); ES HRMS Calcd. for $C_{29}H_{47}NO_{19}NaS$ 768.2361 found 768.2370

1-[3-(2-Aminoethylthio)-propyl (α-D-glucopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside]-2-ethoxycyclobutene-3,4-dione (**109**)

The foregoing amine **86** (12 mg) was treated with diethyl squarate as described for the preparation of **107** to give **109** (10.5 mg, 75%); ^{1}H NMR (600 MHz, D_2O) δ 5.44 (1 H, d, J_{1-2} 3.5, H-1"), 4.89, 4.88 (s, 1 H, H-1'), 4.77, 4.73 (q, 2 H, OCH_2CH_3), 4.71 (s, 1 H, H-1), 4.33 (br s, 1 H, H-2'), 4.24 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.03 (ddd, 1 H, J_{5-6} 2.7, J_{5-6} 4.7, J_{4-5} 10.3 Hz, H-5"), 3.98 (dt, 1 H, J_{gem} 8.1 Hz, J_{vic} 5.3 Hz, OCH_2CH_2), 3.95 (dd, 1 H, J_{gem} 12.4 J_{vic} 2.2 Hz, H-6a), 3.92 (dd, 1 H, J_{gem} 12.4, J_{vic} 2.2 Hz, H-6a'), 3.89 (d, 1 H, J_{gem} 12.5, J_{vic} 1.8 Hz, H-6b"), 3.83 (t, J_{vic} 6.4 Hz, SCH_2CH_2NH), 3.82 (dd, 1 H, J_{2-3}≈J_{3-4} 9.5 Hz, H-3"), 3.76-3.70 (m, 7 H, H-6b, H-6b', H-6b", H-3', H-4', OCH_2CH_2, SCH_2CH_2NH) 3.64 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3), 3.56 (dd, 1 H, H-2"), 3.48 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.44 (dd, 1 H, H-4"), 3.40-3.36 (m, 2 H, H-5, H-5'), 2.83 (t, 2 H, J_{vic} 6.4 Hz, SCH_2CH_2NH), 2.70 (m, 2 H, $CH_2CH_2CH_2S$), 1.95-1.90 (m, 2 H, $OCH_2CH_2CH_2S$), 1.47, 1.45 (t, J 7.1 Hz, OCH_2CH_3) ES HRMS Calcd. for $C_{29}H_{47}NO_{19}SNa$ 768.2361 found 768.2362

1-[3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside]- 2-ethoxycyclobutene-3,4-dione (**110**)

The foregoing amine **86** (6 mg) was treated with diethyl squarate as described for the preparation of **107** to give **110** (5.2 mg, 74%); ^{1}H NMR (600 MHz, D_2O) δ 5.03 (s, 1 H, H-1"), 4.93 (s, 1 H, H-1""), 4.90, 4.89 (s, 1 H, H-1'), 4.77, 4.73 (q, 2 H, OCH_2CH_3), 4.71 (s, 1 H, H-1), 4.40 (br s, 1 H, H-2"), 4.32 (br s, 1 H, H-2'), 4.23 (br s, 1 H, H-2), 4.16 (d, 1 H, $J_{2,3}$ 2.6 Hz, H-2""), 3.99 (dt, 1 H, J_{gem} 10.1 Hz, J_{vic} 5.9, OCH_2CH_2), 3.95-3.91 (m, 4 H, H-6a, H-6a', H-6a", H-6a""), 3.83 (t, J_{vic} 6.4 Hz, SCH_2CH_2NH), 3.78-3.55 (m, 8 H, H-3, H-3', H-3", H-3"", H-4", H-4"",OCH_2CH_2, SCH_2CH_2NH), , 3.50 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.7 Hz, H-4'), 3.49 (dd, 1 H, $J_{3,4}$≈$J_{4,5}$ 9.9 Hz, H-4), 3.40-3.34 (m, 4-H, H-5, H-5', H-5", H-5""), 2.83 (t, 2 H, J_{vic} 6.6 Hz, SCH_2CH_2NH), 2.70 (m, 2 H, $CH_2CH_2CH_2S$), 1.96-1.88 (m, 2H, $OCH_2CH_2CH_2S$), 1.47, 1.45 (t, J 7.1 Hz, OCH_2CH_3); ES HRMS (M+Na) Calcd. 930.2888 found 930.2871

1-[3-(2-Aminoethylthio)-propyl (β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside]- 2-ethoxycyclobutene-3,4-dione (**111**)

The foregoing amine **87** (4.0 mg) was treated with diethyl squarate as described for the preparation of **107** to give **111** (3.1 mg, 71%); ^{1}H NMR (600 Hz D_2O) δ 5.06 (1 H, s, H-1""), 5.02 (1 H, s, H-1"), 5.01 (1 H, s H-1""), 4.96 (1 H, s, H-1""""), 4.91, 4.89 (1 H, s, H-1'), 4.78, 4.73 (q, 2 H, OCH_2CH_3), 4.71 (1 H, s, H-1), 4.39 (2 H, br s, H-2", H-2""), 4.37 (1 H, d, $J_{2,3}$ 3.3 Hz, H-2""), 4.32 (1 H, br s, H-2'), 4.23 (1 H, br s, H-2), 4.08 (1 H, d, $J_{2,3}$ 3.1 Hz, H-2""""), 3.99 (1 H, dt, J_{vic} 6.0, J_{gem} 9.9 Hz, OCH_2CH_2), 3.95-3.91 (m, 6 H, H-6, H-6', H-6", H-6"", H-6"", H-6""""), 3.91 (t, J_{vic} 6.4 Hz, SCH_2CH_2NH), 3.77-3.35 (m, 26 H), 2.83 (t, 2 H, J_{vic} 6.4 Hz, SCH_2CH_2NH), 2.70 (m, 2 H, $CH_2CH_2CH_2S$), 1.96-1.88 (m, 2H, $OCH_2CH_2CH_2S$), 1.47, 1.44 (t, J 7.1 Hz, OCH_2CH_3); EMS (M+Na) Calcd. 1254.4 found 1254.3

1-[3-(2-Aminoethylthio)-propyl (1-thio-β-D-mannopyranosyl)(1→2)(2-deoxy-2-thio-β-D-mannopyranosyl)(1→2)(β-D-mannopyranosyl)(1→2)-β-D-mannopyranoside]-2-ethoxycyclobutene-3,4-dione (**112**)

The foregoing amine **106** (9 mg) was treated with diethyl squarate as described for the preparation of **107** to give **112** (9 mg, 89%); ^{1}NMR (600 MHz, CDCl3) δ 5.17 (s, H-1, H-1"), 5.01 (s, 1 H, H-1""), 4.90, 4.88 (s, 1 H, H-1'), 4.78, 4.73 (q, 2 H, OCH_2CH_3), 4.72 (s, 1 H, H-1), 4.38 (br s, 1 H, H-2'), 4.23 (br s, 1 H, H-2), 4.07 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-2""), 4.00 (dt, J_{gem} 10.1, J_{vic}

5.2, $OCH_2CH_2CH_2$), 3.97-3.89 (m, 4 H, H-6, H-6', H-6",H-3",H-6"), 3.83 (t, J_{vic} 6.3 Hz, SCH_2CH_2NH), 3.81 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-2"), 3.74-3.62 (m, 9 H, H-6, H-6', H-6", H-6"', H-3, H-3', H-3"'OCH_2CH_2, SCH_2CH_2NH), 3.58 (dd, $J_{4,5}$ 9.6 Hz, H-4"'), 3.48 (dd, 1 H, $J_{3,4}$=$J_{4,5}$ 9.5 Hz, H-4), 3.47 (dd, 1 H, $J_{3,4}$=$J_{4,5}$ 9.6 Hz, H-4'), 3.45 (ddd, 4 H, H-5"'), 3.44-3.34 (m, 3 H, H-5', H-5", H-5"'), 3.27 (dd, 1 H, $J_{3,4}$=$J_{4,5}$ 9.6 Hz, H-4"), 2.83 (t, 2 H, J_{vic} 6.3 Hz, SCH_2CH_2NH), 2.70 (m, 2 H, $CH_2CH_2CH_2S$), 1.96-1.88 (m, 2H, $OCH_2CH_2CH_2S$), 1.47, 1.44 (t, J 7.1 Hz, OCH_2CH_3); EMS (M+Na) Calcd. 946.3 found 946.3

Glycoconjugates

Typical procedure for generating protein carbohydrate conjugates: In borate buffer (Na_2BO_4 0.07M, $KHCO_3$ 0.035M, pH 9.5) was dissolved the desired protein (~20 mg/ml) the squarate coupled carbohydrate was then added and the reaction was left for 72 h at room temperature. The reaction was then diluted with deionized water and dialysed in a MICROSEP microconcentrator against 5 changes of deionized water (3ml) where the final volume after concentration was no greater than 500 µL. The solution from the final concentration was lyphophilized to a white solid. MALDI mass spectrometry was preformed on a Voyager elite system from Applied Biosystems at the University of Alberta mass spectrometry facility.

Immunization of experimental animals with Tetrasaccharide-TT conjugate and Hexasaccharide-TT conjugate

Mice

6 female BALB/C mice 9 weeks old were immunized with the tetanus toxoid protein conjugate (5 µg in 100 µL PBS and 100 µL Freunds complete adjuvant, homogenised). On day 13 the mice were subjected to a second immunisation of the protein conjugate (5 µg in 100 µL PBS and 100 µL Freunds incomplete adjuvant, homogenised). On day 22 a trial bleed showed the presence of IgM antibodies in an ELISA using an equivalent carbohydrate linked to BSA. On day 64 the mice were again boosted with the protein conjugate (5 µg in 100 µL PBS and 100 µL Freunds incomplete adjuvant, homogenised). On day 72 a trial bleed showed no antibody against the carbohydrate conjugate or the carrier protein.

Rabbits

2 New Zealand white rabbits where immunized with a tetanus toxiod protein conjugate (50 µg in 500 µL PBS and 500 µL Freunds complete adjuvant, homogenised) in three injections one intramuscularly in rear thigh and two subcutaneously. On day 36 the rabbits were boosted

with (50 µg in 500 µL PBS and 500 µL Freunds incomplete adjuvant, homogenised). On day 59 a third boost with (50 µg in 500 µL PBS and 500 µL Freunds incomplete adjuvant, homogenised) was carried out and on day 70 the rabbits were sacrificed. The sera were then analysed for antibody levels.

Chapter 9

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